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THE SEEDLING RESISTANCE OF WHEAT VARIETIES TO ARTIFICIAL DROUGHT IN RELATION TO GRAIN YIELD[1]

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INTRODUCTION

The ability of a wheat variety to produce a relatively high yield of grain, especially under semi-arid conditions, is of paramount importance if such a variety is to be commercially acceptable. Information on the reaction of varieties to artificial soil drought and the relationship of such reactions to grain yield might be of value, therefore, in breeding programs undertaken to improve the crop. This paper summarizes the results from preliminary experiments on the problem.

The literature on drought resistance in wheat and the relationship of various morphological and physiological characters to grain yield is extensive and will not be reviewed in this paper. The work of Tumanov (3) is of particular interest, however, because the present investigation is largely based on his study. He grew eight varieties of wheat that were sharply contrasted in drought resistance, and studied their respective abilities to endure wilting by means of a pot experiment. The technique followed was to grow the plants under adequate moisture until they reached the "stalking" or shooting stage. At that time water was withheld for two weeks, after which the plants were again watered and the number surviving recorded. The percentage surviving varied from 94% for Tulun 120/32 to 23% for Pusa 4. Caesium, Marquis, and Prelude had survival values of 82, 77 and 49% respectively. He stated that their survival values corresponded, in general, with field reaction.

Commenting on this experiment Maximov (2) states that the results clearly indicate that the capacity to endure prolonged wilting is one of the most important characters, the sum total of which determine drought resistance in plants.

Varietal differences in survival following artificial soil drought have also been reported by Aamodt and Johnston (1). Caesium, which is reported to be drought resistant under field conditions, was found to be more resistant to artificial drought than Reward which is drought susceptible in the field.

EXPERIMENTAL

After several exploratory studies three experiments were conducted. These will be reported separately and designated as A, B and C.

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Experiment A

This experiment was begun in January, 1940, and was conducted in the following manner: Seven varieties of wheat were grown in 1-gallon, glazed crocks each filled with 5 kilos of well mixed, air dried soil. This soil is classified[3] as Haverhill loam. The moisture holding capacity is 24% and the wilting point 9%. Eight plants were established in each crock. There were six replicates (crocks) of each variety in each treatment and these were arranged on the greenhouse bench as randomized blocks.

At the beginning of the experiment the soil was brought to a 15% moisture content and maintained at that level until the plants reached the 3-leaf stage, after which no further water was added. The date at which the plants were permanently wilted was determined, as closely as possible, by means of a humidity chamber. The date of permanent wilting is difficult to determine in wheat and accurate only within limits of 3 or 4 days. Starting from the point of permanent wilting the plants were exposed to four drought treatments. One series was left without water for 5 days, and the others for 7, 12, and 19 days respectively. At the end of each treatment the plants were watered and later the number of surviving plants in each crock was recorded.

A summary of the results is presented in Table 1. Periods of drought up to and including 7 days from the point of permanent wilting caused practically no killing, a 12-day period caused some killing, and by 19 days killing was severe. This would indicate that a treatment of about 15 days would be most satisfactory in studying varietal differences.

TABLE 1.—PERCENTAGE SURVIVAL OF WHEAT VARIETIES WHEN EXPOSED TO ARTIFICIAL SOIL DROUGHT FOR VARIOUS PERIODS OF TIME

Variety	Percentage survival				
	Days of drought from point of permanent wilting				
	5	7	12	19	Ave. 12 and 19
Milturum	98	100	100	54	77
Thatcher	98	100	91	22	56
Hope	100	100	90	22	56
Reliance	98	100	98	19	58
Reward	100	90	48	10	29
Regent	94	96	48	2	25
Renown	98	94	65	0	32
Average	98	97	77	19	48

Considering the 12- and 19-day treatments only: The F value for variety variance divided by error variance = 15.16. 1% point = 3.10. The F value for variety × treatment variance divided by error variance = 1.87. 5% point = 2.24. The minimum significant difference between any two varital averages is 14%.

The data from the 12- and 19-day treatments were analysed by variance. The mean square due to varieties was found to exceed significantly that due to error and also that due to variety-treatment interaction. The mean

[3] Information on the soil characteristics was supplied by Mr. J. Lehane of the Dominion Soil Research Laboratory, Swift Current.

square for variety-treatment interaction did not exceed significantly that due to error. Thus varietal differences were established and these differences were relatively similar in each drought treatment.

Experiment B

In June 1940 this experiment was initiated to test the reaction of 38 varieties to artificial drought. It was carried out in the same manner as Experiment A, except that 12 plants were established in each crock; only one treatment, a 15-day drought period, was used; and an attempt was made to control evaporation from the soil surface. The necessity for this latter change was shown by the fact that unplanted crocks, placed in various parts of the greenhouse, lost varying amounts of moisture by evaporation. Such differential evaporation could be expected to contribute materially to the error of the experiment. The method of control adopted was to fit a cover made of light cardboard, which had been previously dipped in hot wax, to each crock. The cover was slotted to accommodate the plants, and sealed to the unglazed edge of the crock. Cotton-batting was pressed in the spaces between the edges of the slot and the plants. These covers were not entirely satisfactory. Their use materially increased the labour involved and, owing to a combination of high temperatures and vapour pressure, it was difficult to maintain a seal between the cover and the crock. However, they did reduce differential evaporation to a negligible factor.

A summary of the results obtained is presented in Table 2. Varietal differences were established. The magnitude of these differences was very great, ranging from almost complete survival to complete killing. It was also noted that the rate of recovery varied. In general those varieties

TABLE 2.—PERCENTAGE SURVIVAL OF WHEAT VARIETIES WHEN EXPOSED TO A 15-DAY ARTIFICIAL DROUGHT PERIOD AND YIELD FROM TEST PLOTS IN PERCENTAGE OF MARQUIS

Variety	Percentage survival*	Yield in percentage of Marquis	Variety	Percentage survival	Yield in percentage of Marquis
Bena	87	122	Milturum	33	102
Nabawa	81	124	Renown	33	93
Red Fife	79	93	I-28-60 × Milturum H-29-37	22	107
Thatcher	76	114	Pelissier	15	102
Komar	74	116	C.T. 129	12	102
Supreme	62	98	C.T. 211	12	99
Reliance	57	112	Kubanka	12	98
Pearl	56	91	Reward	3	84
Canus	55	112	S.C. Hy. 26-268	3	83
Red Bobs	53	98	T. 0283	0	111
Comet	53	117	R.L.988 × Thatcher H-621	78	—
Marquis	50	100	R.L.988 × Thatcher H-624	72	—
Rival	44	107	R.L.988 × Thatcher H-632	67	—
C.T.217	44	95	R.L.988 × Thatcher H-647	67	—
Hope	43	89	R.L.988 × Thatcher H-538	55	—
Regent	43	104	R.L.988 × Thatcher H-705	53	—
Caesium × Marquis H-37-30	40	102	Iverday	44	—
Apex Sel.	39	102	R.L.988 × Thatcher H-681	39	—
Apex	36	102	T. rigidum	0	—

* The minimum significant difference between any two varieties is 19%.

with little injury recovered much more rapidly than those with severe injury. Bena and Nabawa were outstanding in their ability to recover from injury in a very short time.

Yield data obtained at Swift Current or at the substations in this area were available for 30 of the varieties tested. The yield of each variety was the average of at least three, and usually five or more tests. As all varieties were not grown in the same tests and the same years it was necessary to put the yields on a percentage of Marquis basis in order to have them comparable. As the yield of Marquis was not unduly high or low in any of the tests it is thought that putting the yields on this basis did not introduce any great amount of error. These yield data are also presented in Table 2. A coefficient of correlation was calculated between yield and percentage survival. A significant value of 0.510 was obtained.

Experiment C

In this experiment the reaction of 36 varieties to artificial drought was studied. This set of varieties consisted largely of hybrid lines developed by the Field Crops Department of the University of Alberta in their program of breeding spring wheats for drought resistance. Two tests were made. The first was started in November, 1940, and the second in January, 1941. Each test was conducted in the same manner as Experiment B, except that eight replicates were used instead of six and instead of cardboard covers on the crocks a layer of reasonably fine, air-dried sawdust about two inches deep was placed on the soil surface. In other experiments the sawdust was found to be as effective as the cardboard in reducing evaporation. Its use materially reduced the labour involved.

A summary of the results obtained is presented in Table 3. Varietal differences were again established. The general survival level was much lower in the first than in the second test. This difference is likely due to inaccuracy in determining the point of permanent wilting. Thus, in the first test the actual point of permanent wilting was probably earlier than it was judged to be, and in the second test later. A significant interaction between varieties and tests was obtained. This shows that the relative reaction of the varieties in the two tests was different. The interaction is due largely to the differential reaction of six varieties. Milturum, Supreme and I-28-60 × Milturum (H-39-33) were relatively much more susceptible in the second test than they were in the first, whereas Caesium, Caesium × Marquis (H-29-77) and Caesium × Marquis (H-39-17) were relatively much more resistant in the second test than they were in the first.

Such differential reactions are important in experiments of this nature because, if sufficiently numerous, they tend to render the results inconclusive and may be a serious source of error in drawing conclusions from samples of data in which they are not evaluated.

Yield data on these varieties have been supplied by the Field Crops Department of the University of Alberta. A summary of these is also presented in Table 3. Coefficients of correlation were calculated between the mean percentage survival and mean yield for the four station-years (N36) and between survival and yield for the eight station-years (N22). The values obtained were 0.518 and 0.513 respectively. These values are statistically significant.

TABLE 3.—PERCENTAGE SURVIVAL OF WHEAT VARIETIES EXPOSED TO A 15-DAY ARTIFICIAL DROUGHT PERIOD AND YIELD IN BUSHELS PER ACRE FROM TEST PLOTS

	N.S.N.	Plant survival percentage			Yield bus./acre	
		1st test	2nd test	Ave.	4 test ave.*	8 test Ave.†
I-28-60 × Milturum	H-39-43	73	93	83	27.5	—
I-28-60 × Milturum	H-29-47	76	83	80	27.8	29.2
Milturum	I-28-14	87	67	77	27.4	26.4
Canus	S-34-1	72	82	77	28.0	29.2
I-28-60 × Milturum	H-39-33	77	75	76	29.1	—
Thatcher	I-34-15	65	85	75	24.0	27.0
Apex	I-36-102	57	77	67	22.1	23.4
I-28-60 × Milturum	H-29-35	54	76	65	25.3	27.2
Caesium × Marquis	H-37-24	50	78	64	23.2	25.0
Marquis	I-0-9	57	63	60	24.8	26.2
I-28-60 × Milturum	H-29-37	57	63	60	25.0	26.4
Red Bobs	I-0-18	41	75	58	24.4	—
Caesium × I-28-65	H-39-6	37	75	56	24.1	—
Canus × Caesium	H-39-27	44	66	55	23.5	—
Caesium × Marquis	H-37-30	46	63	54	25.2	26.9
Reward × Caesium	H-37-41	45	63	54	22.2	24.1
Caesium × Marquis	H-37-20	44	59	52	24.2	25.8
Canus × Caesium	H-39-28	45	57	51	21.9	—
Regent	I-36-135	31	71	51	22.6	23.4
Reward × Caesium	H-37-53	32	70	51	22.6	24.6
Caesium	I-28-20	23	78	50	28.0	28.2
Reward × Caesium	H-37-50	33	66	50	23.6	26.0
Supreme	I-40-1	53	47	50	28.3	—
Caesium × Marquis	H-29-77	22	74	48	24.8	26.7
Caesium × Marquis	H-37-9	34	62	48	21.0	22.6
Caesium × Marquis	H-39-9	30	66	48	22.2	—
Caesium × Marquis	H-39-12	32	63	48	23.6	—
Reward × Caesium	H-37-42	20	73	46	26.8	28.3
Caesium × Marquis	H-39-19	41	50	46	22.8	—
Caesium × Marquis	H-37-14	38	50	44	22.6	23.6
Caesium × Marquis	H-39-17	22	65	44	24.4	—
Caesium × Marquis	H-39-26	36	50	43	23.5	—
Caesium × Marquis	H-39-7	19	63	41	24.9	—
Caesium × Marquis	H-39-23	32	49	40	25.8	—
Reward × Caesium	H-37-52	27	53	40	21.8	25.4
Caesium × Marquis	H-37-5	17	46	32	22.7	23.2

The F value for variety variance divided by error variance is 6.42. 1% point = 1.53.
The F value for variety variance divided by variety × test variance is 2.36. 1% point = 2.29.
The F value for variety × test variance divided by error variance is 2.72. 1% point = 1.53.
The minimum significant difference between the average percentage survival is 15%.
* Average, 1940, at Castor (grown on fallow and on stubble), Scott and Swift Current.
† Average 1939–1940 at the above Stations.

DISCUSSION

An examination of the correlation surfaces revealed that those varieties tending to fall outside the swarm could be placed in one of three classes.

1. Those whose survival values are in doubt. An example is Caesium which gave a high survival value in one test and a low value in the other (Table 3). Another example is H-28-60 × Milturum, H-29-37, which had high survivals in the tests reported in Table 3 but a low value in the test reported in Table 2.

2. Those whose yields are in doubt. For example the yield of Thatcher in the first yield column of Table 3 is much lower in relation to such varieties as Marquis and Canus than is usually the case. Its position on the surface is out of line because of its low yield. Another example is the yield of

T.0283 reported in Table 2. This yield is a 3-year average. In one of these years T.0283 yielded less than Marquis; in another it yielded about the same, while in the third year it yielded much more than Marquis or any other variety in the test. In this latter year it escaped severe drought because of its extreme earliness, whereas the later varieties did not. The yield as reported therefore does not represent the inherent yielding ability of the variety.

3. Those that appear to be definitely out of line, such as Hope, with a relatively high survival and a low yield. Not many of the varieties fell into this category.

The magnitude of the coefficients of correlation that were obtained suggest that artificial drought tests would be useful in eliminating low yielding plants or lines from hybrid populations. If the technique used in conducting such tests can be refined so as to reduce materially the error of the determinations the method will be more useful. It should also be kept in mind that the varieties tested in these experiments were, for the most part, rigorously selected for yielding ability. In most unselected hybrid populations it is reasonable to assume that greater differences would have been obtained and that it would be easier to locate low yielding types. However, it is quite possible that artificial drought tests may be of no value in crosses involving varieties that react in a differential manner or whose genetical make-up is such that survival and yield are not positively correlated.

Studies are under way on a number of populations in which the utility of artificial drought tests in actual breeding practice is being investigated.

SUMMARY

The reaction of wheat varieties to artificial drought in the seedling stage was studied in three greenhouse experiments:

A. Seven varieties were grown, under normal moisture conditions, until they reached the 3-leaf stage. Moisture was then withheld until the plants had permanently wilted and for periods of 5, 7, 12, and 19 days thereafter. Periods of drought up to and including 7 days caused practically no killing; a 12-day period caused some killing, and by 19 days killing was severe. Considering only the latter two treatments it was found that varietal differences existed and that these differences were relatively similar in each treatment.

B. The reaction of 38 varieties was studied using only one treatment, a 15-day drought period. Varietal differences were established. Yield data from varietal tests were available for 30 of the varieties included in this test. The coefficient of correlation between percentage drought survival and mean yield was 0.510.

C. Two tests were made on the reaction of 36 varieties. Significant varietal differences were found to exist but the interaction between varieties and tests was also significant. Yield data were available for four station-years on the 36 varieties and for eight station-years on 22 of them. Coefficients of correlation between mean percentage drought survival and yield were 0.518 and 0.513 respectively.

It is suggested that artificial drought tests would be useful in eliminating low yielding lines or plants from hybrid populations.

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RESPONSES OF TOBACCO SEEDLINGS TO CHEMICAL GROWTH SUBSTANCES[1]

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That plant growth is stimulated by chemical growth substances has been reported by various investigators and the literature concerning hormones and growth-promoting substances has become so voluminous that a comprehensive survey of it is beyond the scope of a short paper. However, some papers which deal with the significance of growth substances in the tobacco plant may be cited.

Studies on the growth hormone content of *Nicotiana* by Avery (1) have shown that the concentration of growth substance is greatest in young leaves, decreasing with maturity. The concentration is low at the apex of the leaf, increasing towards the base; thus, there is a concentration gradient from the distal end to the proximal end of the leaf. Higher concentrations of the growth substance in the proximal end of the midrib are especially evident toward the end of the growth period where it is correlated with longitudinal growth (polarized growth) at the basal end of the leaf. Hence it is indicated that the growth in length of the midrib and lateral veins is a factor in the development of the normal leaf pattern. Avery, Burkholder and Creighton (2) have determined that nutrients which favour the normal growth of the tobacco plant also favour the production of growth hormone. Of the six essential elements investigated, namely, nitrogen, sulphur, phosphorus, calcium, potassium, and magnesium, nitrogen was the most important in the production of growth hormone. Nitrogen starvation resulted in a gradual reduction of the growth hormone concentration in the plant; when growth ceased, no hormone could be recovered from the plant. The addition of nitrogen to the nutrient solution stimulated growth and the production of hormone; but, when heteroauxin was added to the nitrogen-deficient nutrient solution, it had no effect on growth.

The present investigation was conducted to make a quantitative and qualitative study of the effect of indole-3-acetic acid (heteroauxin) and naphthyl-acetic acid on the growth of tobacco plants during the seedling period.

MATERIALS AND METHODS

Nicotiana tabacum L. var. Resistant Havana 211 was grown in pots. The seeds were planted in a regular pattern and at a uniform depth. The pots were 6 inches in diameter, 4 inches deep and contained 1,100 grams of air-dry soil. The plants were grown under normal greenhouse conditions and all pots were supplied with the same quantity of water. The pots were rotated horizontally through an angle of 180 degrees daily to nullify the effect of stronger illumination on one side. Fifty seeds were planted in each pot, but, after germination, the number of seedlings was reduced to 30.

[1] Contribution from the Tobacco Division, Experimental Farms Service, Dominion Department of Agriculture, Ottawa, Canada.
[2] Agricultural Assistant.

One pot constituted an experimental group. As criteria of growth, measurements were made at 7-day intervals of the length of the stem from the soil to the origin of the cotyledons and weights of the plants including both tops and roots were recorded at the transplanting stage. Other responses were recorded regularly.

Various methods of applying the growth substances were tested to determine the method of application giving best results.

(A) The total amount of growth substance in aqueous solution was added to the soil at one application in the following concentrations: 0, 0.1, 2.5, 5, 10, 15, 25, and 50 milligrams per pot.

(B) Fifty cc. of aqueous solution of growth substance were added at 3-day intervals for six weeks at the following concentrations: 0, 1/100, 1/10, 1, and 2.5 p.p.m.

(C) The growth substance was dusted on the seed prior to planting (using red copper oxide as a carrier) in the following concentrations: 0.75, 1.5, 3.0, 5.0, 7.5, 10, 12.5, 15, 25, and 50 p.p.m., the application being expressed in parts of the growth substance per million parts of the seed, both by weight. The control plants were grown from seeds dusted with red copper oxide only.

TABLE 1.—SERIES A. HEIGHT AND DRY WEIGHT OF PLANTS TREATED WITH AN AQUEOUS SOLUTION OF INDOLE-3-ACETIC ACID ADDED TO THE SOIL IN ONE APPLICATION

Indole-3-acetic acid per pot	Av. height after 28 days	Percentage increase in height	Av. dry wt. at transplanting stage	Percentage increase in dry wt.
mg.	cm.	%	gm.	%
None, control	5.0		0.120	
0.1	7.0	40	0.150	25.0
2.5	6.8	36	0.130	8.3
5.0	6.2	24	0.100	−16.7
10.0	7.0	40	0.105	−12.5
15.0	6.1	22	0.065	−45.8
25.0	5.3	6	0.050	−58.3
50.0	(Dead)	—	—	—

TABLE 2.—SERIES B. HEIGHT AND DRY WEIGHT OF PLANTS TREATED WITH 50 CC. AQUEOUS SOLUTION OF INDOLE-3-ACETIC ACID ADDED TO THE SOIL AT THREE-DAY INTERVALS

Concentration indole-3-acetic acid	Av. height after 28 days	Percentage increase in height	Av. dry wt. at transplanting stage	Percentage increase in dry wt.
p.p.m.	cm.	%	gm.	%
None, control	5.0		0.120	
1/100	5.1	2.0	0.160	33.3
1/10	5.2	4.0	0.140	11.7
1.0	5.2	4.0	0.145	12.1
2.5	5.5	10.0	0.160	33.3

TABLE 3.—SERIES C. HEIGHT AND WEIGHT OF PLANTS TREATED BY DUSTING GROWTH SUBSTANCE ON SEEDS

Treatment	Av. height after 28 days	Percentage increase in height	Av. dry wt. at transplanting stage	Percentage increase in dry wt.
	cm.	%	gm.	%
Control, no growth substance	5.0		0.120	
0.75 p.p.m. indole-3-acetic acid	5.1	2.0	0.125	4.2
1.50 p.p.m. indole-3-acetic acid	5.1	2.0	0.180	50.0
3.00 p.p.m. indole-3-acetic acid	4.9	−2.0	0.145	20.8
1.50 p.p.m. indole-3-acetic acid + 1.50 p.p.m. naphthyl-acetic acid	4.8	−4.0	0.145	20.8
5.00 p.p.m. indole-3-acetic acid	4.8	−4.0	0.162	35.0
2.50 p.p.m. indole-3-acetic acid + 2.50 p.p.m. naphthyl-acetic acid	4.9	−2.0	0.095	−20.8
7.50 p.p.m. indole-3-acetic acid	4.9	−2.0	0.140	16.7
10.00 p.p.m. indole-3-acetic acid	5.2	4.0	0.145	20.8
12.50 p.p.m. indole-3-acetic acid	5.5	10.0	0.185	54.2
15.00 p.p.m. indole-3-acetic acid	5.0	0.0	0.150	12.5
25.00 p.p.m. indole-3-acetic acid	4.6	−8.0	0.105	−12.5
50.00 p.p.m. indole-3-acetic acid	4.6	−8.0	0.145	20.8

RESULTS AND DISCUSSION

Growth of Stems in Length and Dry Weights

The mean measurements of hypocotyl length at the end of 28 days and mean dry weights at the transplanting stage are presented in the accompanying tables. The greatest height occurred in series A in which the total amount of heteroauxin was added to the soil in one application. The application of 0.1 mg. heteroauxin resulted in a mean increase in height of 40% and an increase in dry weight of 25% as compared to the control plants. Above this concentration, a progressive increase in heteroauxin resulted in a fairly progressive decrease in height, reaching the level of the control plants at 25 mg. (Figure 1, A 1). Root development showed the same trend. Higher concentrations also resulted in a significant decrease in dry weight, the growth of both tops and roots being inhibited. The application of 50 mg. resulted in extreme reduction in the rate of growth, all plants being dead by the 28th day.

The addition of heteroauxin at 3-day intervals (series B) accelerated the rate of growth, as measured both by length of stems and dry weights, without any deleterious effects over the whole concentration range. The increased root development is shown in Figure 1, B. Greatest growth was attained by the 2.5 p.p.m. group which showed a mean increase in height of 10% and a mean increase in dry weight of 33.3% as compared to the control plants.

In series C which received the seed treatment, no significant difference in height was found between the control and the treated groups upon the addition of growth substance over the range 0.75 to 10 p.p.m., but the addition of 12.5 p.p.m. heteroauxin resulted in an increase in height of 10%. The height of the 15 p.p.m. group was at the level of the control, while the 25 and 50 p.p.m. groups each showed a decrease in height of 8%

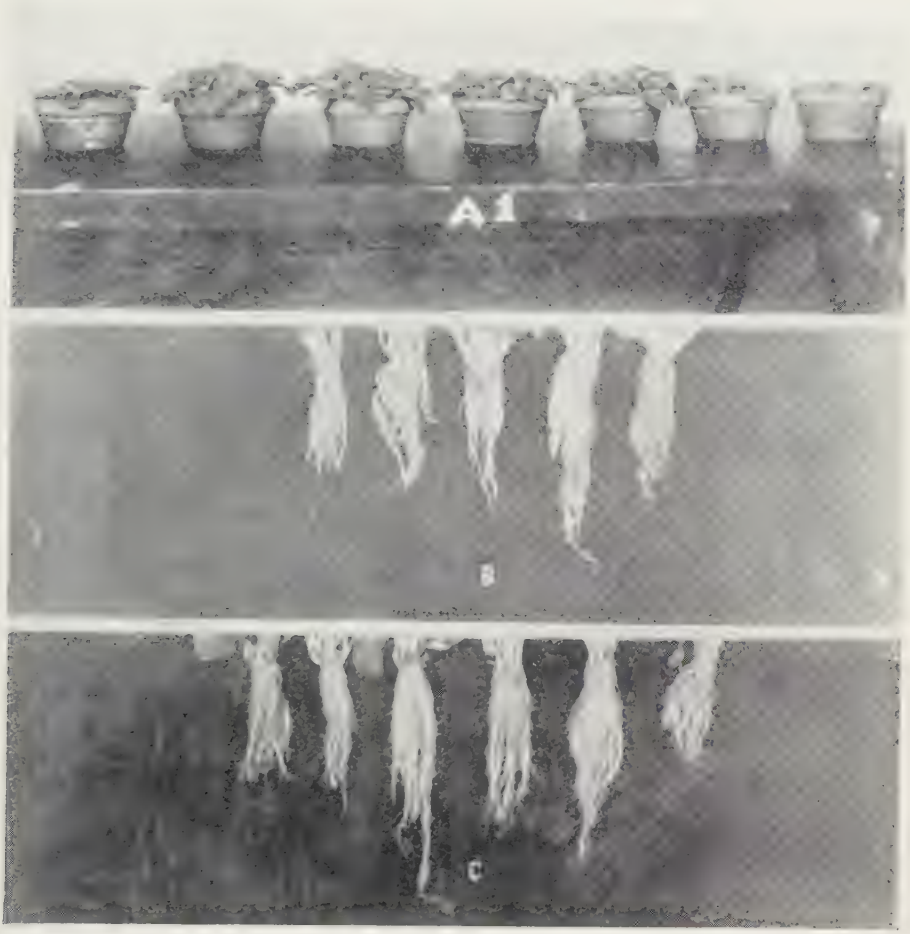

FIGURE 1. A1: Tobacco seedlings treated with heteroauxin added to the soil 10 days after sowing in the following amounts: (left to right) 0, 0.1, 2.5, 5, 10, 15, and 25 mg. per pot. B: Roots of tobacco seedlings treated with 50 cc. aqueous solution heteroauxin per pot added to the soil at three-day intervals for 6 weeks at the following concentrations: (left to right) 0, 1/100, 1/10, 1.0, and 2.5 p.p.m. C: Roots of tobacco seedlings grown from seed on which heteroauxin was dusted in the following concentrations: (left to right) 0, 3, 12.5, 15, 25, and 50 p.p.m. All photographs taken 6 weeks after sowing.

as compared to the control. However, at the transplanting stage, it was found that a significant increase in dry weight resulted from the addition of from 3 to 50 p.p.m. growth substance, excepting the 25 p.p.m. group. Maximum growth by this criterion was found in the 12.5 p.p.m. group which showed an increase of 54.2%. Root development was stimulated by all treatments above the 1.5 p.p.m. concentration (Figure 1, C).

Other Responses

In series A, groups which were treated with 0.1 and 1.25 mg. per pot did not manifest any observable qualitative differences. Higher concentrations resulted in mild to severe epinastic curling of the cotyledons and bending of the hypocotyl during the early growth period, but normal growth was resumed later in all but the 50 mg. group which died.

Treatments in series B and C did not exert any deleterious effects upon the plants and no qualitative differences were observed.

GENERAL DISCUSSION

The evidence seems conclusive that the growth rate of tobacco seedlings can be accelerated by the application of heteroauxin to the soil in which the plants are growing or by the application of heteroauxin and naphthyl-acetic acid to the seed prior to sowing. A favourable response is obtained by the addition to the soil of an aqueous solution of heteroauxin at the rate of 0.5 mg. per sq. ft. at one application 10 days after sowing, or by the addition to the soil at 3-day intervals of 250 cc. aqueous solution per sq. ft. at a concentration of 2.5 p.p.m., the initial application being made just subsequent to planting. While dusting the growth substance on the seed is an effective means of accelerating growth, it does not seem to offer the best method of application, as the optimum concentration, which may vary with variations in environmental conditions, is difficult to gauge and any disparity therefrom cannot be corrected after sowing. The addition of heteroauxin to the soil at 3-day intervals is probably the most advantageous method of application as such low concentrations as are used in this method do not exert any deleterious effect upon the plant and the concentration may be altered to meet the requirements of the plants as indicated by the growth response.

SUMMARY

The effect upon tobacco seedlings (*Nicotiana tabacum*) of indole-3-acetic acid at various concentrations was tested by different methods of application: (1) the addition of aqueous solutions to the soil 10 days after sowing; (2) the addition of dilute aqueous solutions at 3-day intervals; (3) dusting on the seed, using red copper oxide as a carrier. Limited tests were made of naphthyl-acetic acid by the third method.

Significant increases in growth as measured by length of hypocotyl and dry weight were produced by all three methods of application. The evidence indicates that adding the growth substance at 3-day intervals provides the most favourable method of application.

In general, it was found that the lowest concentration range (excepting the seed treatment) accelerated the growth rate of both roots and tops. A higher range of concentrations accelerated the growth rate, but produced epinastic curling of the cotyledons and bending of the hypocotyl early in ontogeny. A still higher range resulted in inhibition of growth.

ACKNOWLEDGMENT

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EFFECTS OF WIND EROSION ON THE COMPOSITION AND FERTILITY OF SOME ALBERTA SOILS[1]

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It has been stated that soil erosion is the greatest single factor causing loss of soil fertility. However, the full significance of this statement is not as yet fully realized. In the more humid regions the eroding of the rich top soil by water occasions the greatest loss; in other areas both wind and water removal of the soil are about equally detrimental. However, in southern and central Alberta and in large portions of the treeless plains of western Canada and of the western States, soil erosion by the wind is the more serious of these two.

In this country severe wind erosion was noted in the Indian Head district in Saskatchewan as early as 1887 (14), but was not reported in Alberta until some years later (11), and has occurred periodically since. The area in this province where wind erosion is most prevalent is roughly in the brown and dark brown soil zones, that is, in the southern and eastern portions of the province. It does occur also in the foothills area, in the Lacombe and Edmonton localities, and even as far north as the Peace River district.

The degree of drifting is influenced by such factors as the insufficiency of root fibre in the soil, the use of improper tillage and cultural practices, etc. However, the immediate causes of soil drifting are the occurrence of high winds in conjunction with low precipitation. In this connection the writer tabulated wind data from different points in the province which showed that there was up to twice as much wind in southern and eastern Alberta as in the central portion. The tables further indicated that in the Peace River district in northern Alberta the amount of wind was midway to the higher figure at Lethbridge and the lower at Lacombe. These wind mileages were also compared with those of Goodwell, Oklahoma and Dalhart, Texas, in the so-called "dust-bowl" of the United States. It was interesting to note that the average wind intensity was 20% greater at Lethbridge, Alberta than at either of these other points. The figures also show that in Alberta most of the winds occur in the late fall, winter and early spring months. At this season much of the land is bare, and as a result serious drifting may take place. It was also of interest to note that at Lethbridge and Medicine Hat four-fifths of the prevailing wind was from the west and southwest while at Calgary the prevailing wind direction was from the northwest. In the central and northern points of the province the wind directions were quite variable.

Actual studies on the effects of wind erosion on the chemical and physical composition of the soil are very limited in this country. In 1920 Ellis (10) noted the loss of organic matter caused by wind erosion in the soils of southwestern Manitoba. Various publications of a general nature

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have been issued since. In 1935 Moss (15) reported the results of investigations in Saskatchewan of the effects of wind erosion on the chemical and physical composition of the soil. Papers of a similar nature have been issued by Daniel and Langham (7, 8) of Oklahoma. It is also interesting to note that Waksman (17) has recently presented a paper pointing out the detrimental effects of soil erosion to the microbiological life in the soil. Still more recently an intensive study of the various aspects of soil drifting has been inaugurated at the Soil Research Laboratory at Swift Current, Saskatchewan.

MATERIAL AND METHODS

The soil samples used in this project were composite samples collected from various locations in southern and eastern Alberta in the summers of 1937, 1938, and 1939, during the course of the soil survey under the P.F.R.A. In all, 38 locations were sampled, covering areas in the brown, dark brown and shallow black soil zones. Ten were from coarse textured soils (fine sands and fine sandy loams), 16 medium textured soils (loams and silt loams), and 12 fine textured soils (clay loams and clays), making in all 114 soil samples. In addition, 6 samples of dust were collected in buildings in proximity to locations from which the soil samples were taken. At each location a composite sample was taken of *(a)* the normal (relatively free from drifting), cultivated, surface soil to a depth of 6 inches, *(b)* the accumulated drift, *(c)* the surface after wind removal or what is termed the "exposed subsurface." All samples, with one exception, were taken from cultivated fields or the drift accumulations from cultivated fields. This was done to reduce the possibility of variation due to losses caused by cultivation and cropping as pointed out in a recent paper by Caldwell *et al.* (6). Further, each group of three was collected within a radius of 50 yards to minimize soil variability.

In addition to this, it was recorded in the writer's field notes that the coarse textured soils from which the samples were taken had drifted moderately to severely, with the majority severely drifted. The medium textured soils exhibited from slight to moderate drifting, with only one case of severe drifting. In regard to the fine textured soils, the drifting ranged from moderate to severe, though mostly of the moderate order.

The degree of drifting occurring at the individual locations would undoubtedly have considerable bearing on the results obtained. However, it was the writer's purpose to compare the effects of wind erosion on different soil types or textures as they occurred under field conditions.

A preliminary pot culture experiment was carried out in a greenhouse to observe how growing plants developed on eroded and non-eroded soils, under a similar set of conditions.

All of the soil and dust samples were analyzed for total nitrogen and for organic carbon and then the C : N ratio was calculated from these results. The nitrogen was determined by the official Gunning-Hibbard method, and the duplicates checked to 0.009% of each other. The organic carbon was determined by Schollenberger's chromic acid method as modified by Allison (1) and duplicates were checked to within 0.04% of each other. Total organic matter was then calculated by using the generally recognized factor of 1.724.

Mechanical or physical analyses of all the soil and dust samples were carried out by the improved Bouyoucos hydrometer method (2,3) which has been shown to be quite accurate in comparison with the pipette method (4). The percentages of silt and clay were determined according to the new standards of the United States Bureau of Soils.

RESULTS AND DISCUSSION

Pot Experiment

Twenty-four 6-inch clay pots were used in this experiment, each soil sample being tested only in duplicate due to insufficiency of sample. In all cases 1750 grams of air-dry soil were used. The soil samples consisted of the normal surface, the accumulated drift and the exposed subsurface samples of fine sand, loam, silt loam and clay collected from drifted areas in southern Alberta. Five kernels of Reward wheat were planted in each pot and after their emergence the plants were thinned to three. Then the pots were kept in a greenhouse at as near as possible to optimum moisture conditions, and the temperature maintained at 60 to 70°F. Artificial lighting was used from dusk until midnight to offset the short winter days. After 107 days, the plants were cut, weighed, dried at room temperatures, and reweighed. As the plants had advanced only to the soft dough stage, the total weight rather than the weight of the grain itself was taken as an index of the relative productivity of the drifted and undrifted soils.

No differences were evident in the dates of emergence of the wheat on the eroded and non-eroded soils themselves but there was a lag of 4 days in the germination of the wheat on clay soils. However, by 6 weeks a marked difference was observed in the size of plants on the eroded and non-eroded fine sand (Figure 1). In 11 weeks, i.e., after the plants were headed, the differences were further accentuated on this soil type (Figure 2). Actual measurements indicated that the wheat on the normal, undrifted, fine sand reached 40 inches in height, on the accumulated drift only 25

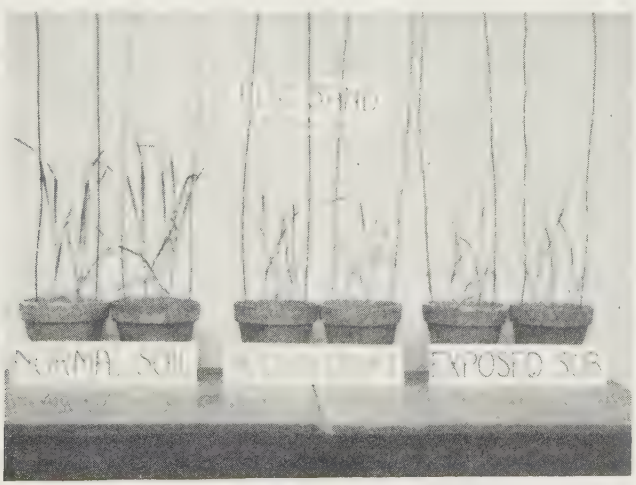

FIGURE 1. Pot culture experiment at 6 weeks, showing wheat on some normal and eroded Alberta soils.

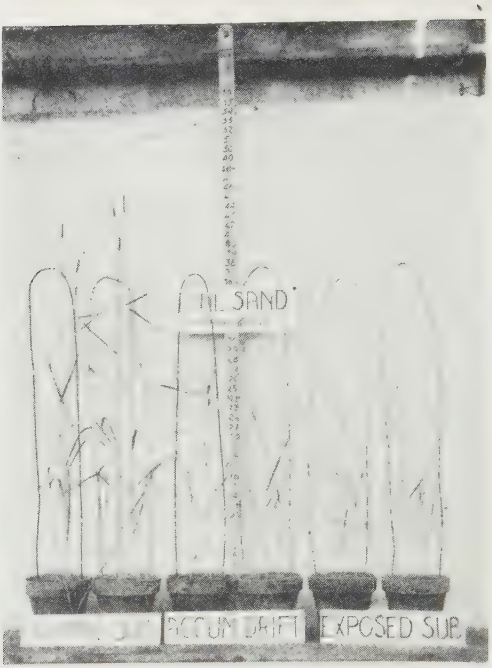

FIGURE 2. Pot culture experiment at 11 weeks, showing wheat on some normal and eroded Alberta soils.

inches, and those on the exposed subsurface were only 18 to 20 inches. There was also a striking difference in the size of heads, those on the drifted fine sand being only about one-half as long as those on the normal fine sand. It was subsequently shown also that out of the 3 heads formed in each pot of fine sand, 2 were fertile in each of the normal soil pots, but they were all sterile in the remaining 4 pots containing the accumulated drift and the exposed subsurface samples. This sterility, though perhaps influenced by seasonal or light factors, has been noted by Griffiths (13) in Australia under actual field conditions on badly eroded soils. However, on the clay soil only half of the spikes were fertile on both the normal and eroded soils. On the loam and silt loam pots practically all the heads formed were fertile. The complete results appear in Table 1.

The data in Table 1 show that there is in general good agreement in the different sets of duplicates, both in regard to the total weights of the plants and in the numbers of heads formed. The plants growing in the eroded and non-eroded fine sand show striking variations. The average total weight of the plants on the accumulated drift is less than half of that on the normal soil, while that on the exposed subsurface is only one-fifth of the normal, indicating a very serious impairment in productivity on this soil type. The results on the silt loam follow a similar trend but the differences are less striking. On the loam soil used the average air-dry weights of the wheat grown on the normal and eroded samples are identical. The clay soils produced similar weights of plants on both the normal surface soil and the exposed subsurface sample, but those grown on the accumulated drift sample were somewhat heavier. Analyses of the wheat plants grown

TABLE 1.—TOTAL WEIGHTS AND NUMBERS OF HEADS ON WHEAT PLANTS IN POT CULTURE EXPERIMENT WITH SOME NORMAL AND ERODED ALBERTA SOILS

Pot No.	Soil used	Wt. of plants in grams				No. of heads	
		Green wt.	Ave.	Air-dry	Ave.	Total	Fertile
1	Fine sand—Normal	7.5		3.2		3	2
2	Normal	8.5	8.0	3.5	3.4	3	2
3	Acc. drift	3.5		1.5		3	0
4	Acc. drift	2.5	3.0	1.2	1.4	3	0
5	Exp. sub.	1.3		0.8		3	0
6	Exp. sub.	1.2	1.3	0.6	0.7	3	0
7	Silt loam—Normal	22.0		7.5		4	3
8	Normal	20.5	21.3	6.9	7.2	5	3
9	Acc. drift	19.5		6.7		3	3
10	Acc. drift	18.0	18.8	5.8	6.3	3	3
11	Exp. sub.	11.5		4.0		3	3
12	Exp. sub.	13.5	12.5	4.8	4.4	3	3
13	Loam—Normal	12.5		4.2		3	3
14	Normal	12.0	12.3	3.5	3.9	3	2
15	Acc. drift	12.0		3.9		3	2
16	Acc. drift	11.5	11.8	4.0	4.0	3	3
17	Exp. sub.	13.0		4.2		3	2
18	Exp. sub.	10.5	11.8	3.4	3.8	3	1*
19	Clay—Normal	28.0		8.7		7	3†
20	Normal	25.5	26.8	7.1	7.9	6	3
21	Acc. drift	37.5		10.0		8	4†
22	Acc. drift	35.0	36.3	9.7	9.9	9	3
23	Exp. sub.	30.5		8.6		7	3†
24	Exp. sub.	25.5	28.0	7.6	8.1	7	2

* In this case 2 unclassified heads removed by a mouse.
† In this case 1 unclassified head removed by a mouse.

on these eroded and non-eroded soils showed that there was no correlation between percentages of nitrogen in the wheat plants on the drifted or undrifted soils. However, there was a direct relationship between the percentages of nitrogen in the soil and the total weights of the plants grown thereon.

In summing up this section on the pot experiment, the results indicate that the fine sand soil type has been seriously impaired by wind erosion in so far as actual wheat production is concerned. A smaller, though significant, injury was observed in the silt loam, while the loam and clay soils in this experiment did not exhibit any impairment in productivity.

Mechanical Analyses

Mechanical analyses were conducted on all of the normal, accumulated drift and exposed subsurface samples to determine the effects of wind erosion on the physical composition of the soil. However, a mere comparison of the varying percentages of the soil fractions does not present a complete picture of the situation. According to Bradfield (5), wind erosion or water erosion may destroy the natural soil aggregates, leaving an impervious, deflocculated surface, even though the change in the percentages of sand, silt or clay may be relatively small. Such a physical environment reduces

microbiological activity (17), and in consequence the supply of available plant nutrients is decreased. It is therefore apparent that the physical composition of the soil is of great importance in crop production.

In Table 2 are recorded the average mechanical analyses of the normal and eroded soils arranged in three groups or classes. That is, we have the coarse textured soils, including the fine sands and fine sandy loams; the medium textured soils, or loams and silt loams; and the fine textured soils, or the clay loams and clays.

A table containing the analyses of the 114 individual samples was not included on account of its bulky nature, but it is of interest to compare the limits of variability along with these average figures. The results for the accumulated drifts of the coarse textured soils, as compared to the normal surface soil, show that there has been an increase in sand up to 48.1% with the average increase as shown in Table 2 of 17.0%. All of the individual samples of the accumulated drift showed losses in their silt contents ranging from 1.7% to 80.0%, with an average loss of 50.7%, indicating that the wind has sifted out about one-half of the original silt. The clay content in the accumulations varied from a slight increase of 7.4% to losses up to 46.3%, with an average loss of 26.8%. In the exposed subsurfaces of these coarse textured soils the sand content has varied from a decrease of 8.5% to increases up to 23.5%, or a net increase of 6.5%. The silt fraction has varied from increases in two individual cases to decreases up to 66.9%, or an average decrease for the group of 24.2%. The clay content in the exposed subsurface samples was found to be quite variable, with half of the samples showing increases and the other half decreases, apparently depending on the nature of the underlying subsoil. The net result is an increase of 4.2%. We therefore see that in the movement of the lighter soils by wind, on the average about half of the silt and one-quarter of the clay have been lost.

TABLE 2.—AVERAGE MECHANICAL ANALYSES OF SOME NORMAL AND ERODED ALBERTA SOILS

Location	Sand		Silt		Clay	
	Per cent	Diff., %	Per cent	Diff., %	Per cent	Diff., %
Coarse textured soils (10 locations)						
Normal surface	72.3	—	20.7	—	7.1	—
Acc. drift	84.6	+17.0	10.2	−50.7	5.2	−26.8
Exp. subsurface	77.0	+6.5	15.7	−24.2	7.4	+4.2
Medium textured soils (16 locations)						
Normal surface	47.9	—	40.4	—	11.8	—
Acc. drift	55.5	+15.9	33.3	−17.6	11.2	− 5.1
Exp. subsurface	46.5	− 2.9	39.3	− 2.7	14.2	+20.3
Fine textured soils (12 locations)						
Normal surface	20.5	—	51.1	—	28.3	—
Acc. drift	18.6	− 9.3	51.2	+ 0.2	30.2	+ 6.7
Exp. subsurface	19.6	− 4.4	50.4	− 1.3	29.7	+ 4.9

In this connection the investigations of Daniel (7) in Oklahoma show losses of about three-fifths of the silt and one-half of the clay from the accumulated drifts of coarse textured soils collected from 24 locations. Moss's (15) results with samples of light textured soils collected from 5 locations in Saskatchewan indicate losses of about one-half of the silt and one-third of the clay by wind erosion.

In regard to the medium textured soils there is more variability in the individual samples, the average figures indicating that the losses have been considerably less than in the lighter soils. In the accumulated drifts of these loams and silt loams collected from 16 different locations the sand varies from an increase of 48.4% to a loss of 14.6%, or a net increase of 15.9%. The silt fraction varies from gains up to 11.5% and losses up to 81.5%, but an average loss of only 17.6%, as seen in Table 2. This compares with an average decrease of 50.7% in the silt content of light textured soils. The clay content varies from increases as high as 48.0% and losses up to 33.3%, giving a net loss of 5.1%. In the exposed subsurface samples of these medium textured soils the sand varies from increases to 16.0% and decreases as great as 30.5%, giving an average decrease of only 2.9%. Similarly, the silt fraction shows gains up to 16.0% and losses to 31.6%, or a net loss of only 2.7%. In regard to the clay content we note increases in three-quarters of the individual samples ranging up to 57.7% and, in the remainder, decreases to 21.7%, or an average increase in clay of 20.3%.

On the whole, then, the medium textured soils have been affected considerably less by wind erosion than the light textured soils. Although there has been a significant increase in the sand content and a loss in the silt fraction of the accumulated drifts, the data for the exposed subsurface samples, with which we should be more concerned, indicate only minor changes in sand and silt, but a material increase in clay. This apparent resistance of the medium textured soils to wind erosion has been noted by various authors (9, 18, 19).

In the fine textured soils the sand content of the individual samples ranges from an increase of 16.7% to a decrease of 82.9%, giving an average decrease of 9.3%. The silt fraction varies from a gain of 20.4% to a loss of 10.5%, with the average gain of 0.2%. The clay content also shows gains up to 31.4% and losses to 17.8%, or a net gain of 6.7%. In the exposed subsurface samples of these clay loams and clays the sand content of the individual samples shows great variability, ranging from increases up to 54.5% and decreases to 82.9%, with the net result as given in Table 2 showing a decrease of only 4.4%. The silt fraction varies only from a gain of 16.6% to a loss of 14%, giving a net loss of 1.3%. The clay content also varies from an increase to 30.5% to a decrease of 23.3%, or an average increase of only 4.9%.

It appears therefore that, although there is considerable variability in some of the individual samples of fine textured soils, on the average both the accumulated drift and the exposed subsurface vary only slightly from the surface soil. Since it was noted earlier that on these heavier soils moderate to severe drifting had occurred, it must be assumed that these soils have had a deep, uniform profile, and that the wind has removed the surface soil "en masse", probably in granular form, without sifting out the

silt and clay fractions as in the case of the light and medium soils. These results for the fine textured soils are borne out by Moss (15) who shows that the accumulated drifts from nine different locations are identical to the cultivated surface soil samples from which the drift samples were formed.

In concluding this section on the effects of wind erosion on the physical composition of the soil, several points have been noted:

(1) In the movement of coarse textured soils the wind on the average has sifted out one-half of the original silt and one-quarter of the clay, as indicated by the analyses of the accumulated drift as compared to the normal surface soils.

(2) In medium textured soils the accumulated drifts show, on the average, losses of only one-fifth of the silt and insignificant losses of clay.

(3) The soil moved by the wind from fine textured soils is practically identical in percentage composition of the soil separates to the original cultivated surface soil.

Chemical Analyses

Certain chemical analyses were carried out on the normal and drifted soils to determine the effects of wind erosion on the chemical composition of the soil as an indication of its fertility. The total nitrogen and the organic matter were determined on all of the samples by methods as previously noted. In addition, total phosphorus and acid soluble phosphorus were determined, but on too limited a number to warrant including these results. In Table 3 are listed only the average figures for the nitrogen, organic matter and also the carbon : nitrogen ratios. The variations in the individual results are noted in the discussion.

TABLE 3.—AVERAGE CHEMICAL ANALYSES OF SOME NORMAL AND ERODED ALBERTA SOILS

Location	Nitrogen		Organic matter		C : N ratio
	Per cent	Diff., %	Per cent	Diff., %	
Coarse textured soils (10 locations)					
Normal surface	0.169	—	3.36	—	11.6
Acc. drift	.087	−48.5	1.67	−50.3	11.4
Exp. subsurface	.103	−39.6	1.82	−45.8	10.1
Medium textured soils (16 locations)					
Normal surface	.270	—	5.39	—	11.4
Acc. drift	.237	−12.2	4.79	−11.1	11.7
Exp. subsurface	.225	−16.7	4.43	−17.8	11.4
Fine textured soils (12 locations)					
Normal surface	.283	—	5.00	—	10.1
Acc. drift	.270	− 4.6	4.63	− 7.4	9.8
Exp. subsurface	.247	−12.7	4.15	−17.0	9.7

In the accumulated drifts of the coarse textured soils a loss of nitrogen occurs in each case ranging from 2.9% to 70.5%, or an average loss of 48.5%. Similarly, with the organic matter the losses range from 3.6% to 77.2%, giving an average decrease of 50.3%. In the exposed subsurface, decreases in nitrogen and organic matter also occur in each case in the fine sand and fine sandy loam samples analyzed. For nitrogen the decreases range from 8.6% up to 69.7% or an average of 39.6%, while they varied from 25.3% to 67.4% or an average loss of 45.8% in organic matter. We see, then, that the drift contains on the average one-half of the nitrogen and organic matter as compared to the normal, while in the exposed subsurfaces the decrease is just less than half. These losses may be compared with those of Daniel and Langham (8) of Oklahoma for the analyses of drift from coarse textured soils, who recorded losses of approximately one-third of the nitrogen and organic matter in the accumulated drift as compared to the normal surface soil. Moss (15) in Saskatchewan also found a loss of one-half the nitrogen and organic matter.

In the medium textured soils more variation has occurred in the individual results, but the average losses of both nitrogen and organic mattter are considerably lower. The nitrogen content in the accumulated drift ranges from a gain of 36.0% to a loss of 61.4%, or an average loss of only 12.2% as indicated in Table 3. The organic matter also varies from an increase of 44.5% to a decrease of 55.2%. In both instances gains were recorded in only one-third of the individual samples and losses in two-thirds. In the exposed subsurfaces the variation is from increases in 2 cases of only 7.2% and 8.3%, to decreases ranging up to 55.3% in the other 14, or an average decrease of 16.7%. Similarly the organic matter varies from increases of 15.2% to decreases of 57.2%, or an average decrease of 17.8%. These results indicate that the loss in fertility of medium textured soils is considerably less than in the coarse textured soils as indicated by data on the nitrogen and organic matter contents. In this connection Daniel and Langham (8) and Moss (15) indicated average losses only up to 9.5% in the accumulated drifts of these medium weight soils.

FIGURE 3. Some accumulated drift from clay soil southeast of Macleod, near Stand-Off, Alberta.

Considering the results for the fine textured soils, we note that the average figures are very similar to those of the medium textured soils. The nitrogen content in the accumulated drifts varies from gains of 15.2% to losses, found in most of the individual samples, up to 48.6%, but an average loss of only 4.6%. The organic matter varies similarly from plus 16.2% to minus 53.1%, with an average loss of 7.4% as compared to the normal cultivated surface soil. In the exposed subsurface the nitrogen ranges from increases to 12.3% to decreases (found in three-fourths of the individual samples) up to 59.3%, giving an average decrease of 12.7%. Similarly the organic matter varies from small gains of 1.4% and 7.9% in two instances to losses as high as 65.1%, or an average loss of 17.0%. It appears therefore that, although moderate to severe drifting had occurred on these heavy soils, as pointed out earlier (see Figure 3), yet the actual decreases in nitrogen and organic matter are no greater than in the medium textured soils. Similar results were obtained by Moss, Daniel, and Langham. This would lead one to assume the presence of a deep "A" horizon in the soil profile, and also that very little sortation of the soil particles of these heavier soils was caused by wind erosion. This latter point was also noted in the previous section on the mechanical analyses of these eroded and non-eroded soils.

FIGURE 4. Recent wind erosion on sandy land near Monitor, Alberta. This field was once cultivated.

In regard to the C : N ratios as given in the last column of Table 3, some interesting points are observed. In all three cases the C : N ratio for the exposed subsurface is lower than for the normal, cultivated surface soil. This is in agreement with observations by Salter (16), that with the ageing of organic matter, as occurring in the subsoil, the C : N ratio is narrowed. In regard to the accumulated drift samples, we note that the C : N ratio is slightly lower than that of the normal surface soil, in the light and heavy soils, but slightly higher for the medium soils. This would indicate that the drifting has been more severe on the sandy and clayey soils or that there has been an accumulation of more coarse, undecomposed, organic matter on the medium weight soils, or a combination of both.

In concluding this section on the chemical analyses of some eroded and the corresponding non-eroded soils, several points should be noted:

(1) In coarse textured soils the data indicate that very serious damage has been caused by wind erosion, with the loss of one-half of the nitrogen and organic matter in the accumulated drifts, and slightly less than one-half in the exposed subsurface samples as compared to the normal surface soil (Figure 4).

(2) Data for the medium textured soils indicate that the losses have been much less than for the light soils, with a reduction of approximately 15% in both nitrogen and organic matter.

(3) The actual figures indicate that the losses in fertility of the fine textured soils were of the same order as for the medium textured soils, although it was noted that the drifting had been more severe in these heavy soils.

FIGURE 5. View of approaching dust storm, Taber, Alberta, June 2, 1937. *(Courtesy W. Prowse.)*

Dust Analyses

Six samples of dust from southern Alberta were analyzed in order to determine the composition of that portion of the soil which has been lost by wind erosion. These samples were collected at heights of only 5 to 25 feet, although the dust cloud may rise thousands of feet (see Figure 5). Four samples were collected in a silt loam area and two from a sandy loam area. The average analyses of these samples showed that the dust was just about twice as rich in nitrogen as the surrounding surface soil and nearly 75% higher in organic matter. These figures are very similar to those reported by Fly (12) for the analyses of nitrogen and organic matter in 15 samples of dust in Oklahoma.

In the mechanical analyses of dust it appeared that its composition was independent of its place of origin, the dust from both the silt loam and sandy loam areas being approximately two-thirds silt with lesser amounts of sand and clay.

SUMMARY

In a study made of winds in different parts of Alberta, and of the effects of wind erosion on the composition of Alberta soils, the following points were noted:

(1) The survey of meteorological data indicated that the winds in southern and southeastern Alberta are nearly double those in the central part of the province, and also about 20% higher than at 2 points in the "dust bowl" of the United States. It was also noted that from one-half to four-fifths of the prevailing winds in southern Alberta are from the west and southwest.

(2) The results from the pot culture experiment, carried out with eroded and non-eroded soils, indicated that the fine sand soil has been seriously damaged in so far as actual wheat productivity is concerned. The silt loam samples showed a smaller injury, while the loam and clay soils used in this experiment exhibited no decrease in productivity.

(3) In the mechanical analyses of 114 samples of eroded and corresponding non-eroded soils, the data indicated that the wind had sifted out one-half of the silt and one-quarter of the clay, from the accumulated drifts of the sandy loams and fine sandy loams. In the loams and silt loams the accumulated drifts showed losses of 20% of the silt as compared to the normal samples, and slight losses of clay. The soil moved by the wind in clay loam and clay areas was practically identical in its physical composition to the normal surface soil.

(4) The chemical analyses conducted on these samples indicated very serious losses in fertility in the coarse textured soils, ranging from losses of one-third to one-half of the nitrogen and the organic matter in the accumulated drift and exposed subsurface samples as compared to the normal, cultivated surface soil. In the medium textured soils the losses incurred by wind erosion were only about 15% in nitrogen and organic matter. The fine textured soils showed percentage losses in these two constituents similar to the losses found in the medium textured soils. However, under actual field conditions the fine textured soils appeared to have been eroded more severely than the loams and silt loams.

(5) Analyses of dust samples indicated that they were just about twice as rich in nitrogen and organic matter as the cultivated surface soil from the adjacent area.

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THE RELATION OF SOIL ORGANIC MATTER TO THE PRODUCTION OF FLUE-CURED TOBACCO[1]

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The importance of maintaining an adequate supply of organic matter in the soil for successful crop production can scarcely be over-emphasized, but probably with no crop other than flue-cured tobacco is there as much necessity of maintaining and controlling the soil organic matter within comparatively narrow limits. The natural characteristics which make a soil suitable for growing flue-cured tobacco, such as coarse texture, loose, open structure and good under-drainage, are not conducive to the accumulation of organic matter in the soil and consequently its natural level is quite low in these soils. This natural level has in many cases been lowered further by the combined effects of intensive cropping and wind and water erosion. The necessity of protecting and replenishing the organic matter is becoming increasingly important to the tobacco grower in the maintenance of good yields and uniform quality. Since, however, increasing the organic matter necessitates raising the nitrogen level it can readily be realized that from the standpoint of flue-cured tobacco production the build-up of organic matter can be overdone. The detrimental qualities imparted to the leaf by the release of too much nitrogen throughout the season are manifested by a coarse, dark green leaf which is late in maturing. Consequently, the organic matter content must be accurately controlled and adjusted to suit the needs of the crop.

EXPERIMENTAL PROCEDURE

Much of the experimental work of the Dominion Experimental Substation at Delhi, Ontario, has been directed toward the problem of finding suitable practical methods of maintaining the soil organic matter at a desirable level for flue-cured tobacco production. It is the purpose of this paper to summarize some of the phases of this work as far as the experiments have progressed. The organic matter investigations were carried along with, and were supplementary to, the regular agronomic phases of these experiments, and in this way the results of the soil analyses could be correlated with the data relative to the yield and quality of the tobacco crop. The various treatments were applied on quadruplicate plots arranged in randomized blocks. In the treatments where barnyard manure was applied, the application was made at the rate of 5 tons per acre in the spring before plowing. Each tobacco crop was fertilized with a complete tobacco fertilizer at the rate of 1000 lbs. per acre. The analysis used was a 2–10–8 in all years except in 1935 when a 3–10–6 was used. Immediately

[1] A joint contribution of the Tobacco Division, Central Experimental Farm, Ottawa, Ontario, and the Dominion Experimental Substation, Delhi, Ontario, of the Dominion Experimental Farms Service; and the Department of Chemistry, Ontario Agricultural College, Guelph, Ontario.

Acknowledgment is given to Mr. F. A. Stinson, Officer-in-Charge, Dominion Experimental Substation, Delhi, to Dr. N. T. Nelson, Chief, Tobacco Division, Ottawa, Ontario; and to Mr. G. N. Ruhnke, Professor of Chemistry, Ontario Agricultural College, Guelph, for their advice and administrative support of this work.

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after harvesting the tobacco the cover crops were sown. The cured tobacco leaf was sorted into commercial grades and a numerical expression of its market value was thus obtained.

For the purpose of studying the organic matter content in the soil, samples of surface soil (6 to 7 inches) were taken from the plots periodically throughout the growing seasons, the first series of samples being taken in each case before the fertilizer was applied and the last series taken after the tobacco was harvested. The samples were taken with a soil auger and in order that they might be as representative of the plots as possible, the composite samples were obtained from a large number of borings taken at random from each plot, in accordance with a method previously devised for these conditions (3). The soil was air-dried, sieved and carefully mixed. Organic carbon in the soil was determined by the wet combustion method of Allison (2) and from this was calculated the total organic matter. The active organic matter was determined by the rapid chromic acid digestion method of Thomas and Williams (7). With this method only the less resistant fraction of the organic matter is measured, thus providing an index of the amount of organic matter able to undergo rapid decomposition. This fraction which is proportional to the total amount varies with soil management and cropping practices.

RESULTS AND DISCUSSION

A consideration of the experimental data would suggest that there are factors other than treatment which have a marked influence on the content of organic matter in these soils. One of the replications in the block of plots used in this experiment has suffered from wind erosion to some extent, a fact borne out by several years of consistently low yields in this particular location. Lewis and Hunter (4), working with green manure crops on coastal plain soils, found no definite relationship between the amount of green manure added and the organic matter accumulated during the period under study. However, they reported that in general the largest crop yields were from the plots showing the highest organic matter. Although not without some exceptions the data presented in this paper show the same general tendency. Table 1, A, in which the treatments with tobacco in continuous culture are compared, shows that the most organic matter and the highest yields were obtained where manure was applied and the rye cover crop grown. In the 6-year duration of the experiment the lowest yields were invariably obtained where tobacco was grown every year with no cover crop in the intervals between seasons. This treatment also shows the lowest content of organic matter. In this connection, McKaig *et al.* (5) found in both field plot and lysimeter studies with Norfolk coarse sand, a similar soil type to that involved in these experiments, that a winter cover crop of rye once in three years maintained the organic matter at a higher level than where the soil was continuously winter-fallowed.

In Table 1, B and C, comparisons between continuous tobacco culture and the 2-year rotations in which tobacco was alternated with rye show the latter to be superior in returns per acre. The continuous culture with manure was slightly better in yield than the 2-year rotation without manure, but the better quality of tobacco from the latter treatment resulted in

TABLE 1.—SUMMARY OF RESULTS SHOWING THE EFFECTS OF ROTATIONS, COVER CROPS AND BARNYARD MANURE ON FLUE-CURED TOBACCO

Rotation or treatment	Organic matter season average			Crop data averages	
	Total O.M. %	Active O.M.		Yield per ac. lbs.	Returns per ac. $
		Per cent	Per cent of total		
TABLE A	Season average 1940			Six-year aver. 1935–40 incl.	
Tobacco every year, land left without a cover crop	1.26	.90	71.3	1050	274
Tobacco every year, followed by cover crop of oats	1.43	1.08	74.4	1130	273
Tobacco every year, followed by cover crop of rye	1.33	.98	73.8	1163	297
Tobacco every year with manure, followed by cover crop of rye	1.46	1.11	75.4	1263	310
TABLE B	Season average 1940			Three-year aver. 1936–38 -40	
Tobacco with manure following rye, disced when mature, with rye cover crop	1.38	.92	68.6	1232	286
Tobacco every year with manure, followed by rye cover crop	1.46	1.11	75.4	1096	260
Tobacco following rye disced when mature, with rye cover crop	1.08	.79	73.4	1058	263
Tobacco every year, followed by rye cover crop	1.33	.98	73.8	979	231
Tobacco every year, followed by oat cover crop	1.43	1.08	74.4	950	224
Tobacco every year, land left without a cover crop	1.26	.90	71.3	867	209
TABLE C	Season average 1939			Three-year aver. 1935–37–39	
Tobacco with manure following rye, disced when mature, with rye cover crop	1.39	1.20	86.3	1539	420
Tobacco every year with manure, followed by rye cover crop	1.31	1.18	90.0	1429	361
Tobacco following rye, disced when mature, with rye cover crop	1.52	1.27	83.5	1418	384
Tobacco every year, followed by rye cover crop	1.23	1.08	87.8	1346	363
Tobacco every year, followed by oat cover crop	1.31	1.16	88.6	1301	318
Tobacco every year, land left without a cover crop	1.31	1.12	85.5	1233	335

TABLE 1.—SUMMARY OF RESULTS SHOWING THE EFFECTS OF ROTATIONS, COVER CROPS AND BARNYARD MANURE ON FLUE-CURED TOBACCO—*Concluded*

Rotation or treatment	Organic matter season average			Crop data averages	
	Total O.M. %	Active O.M.		Yield per ac. lbs.	Returns per ac. $
		Per cent	Per cent of total		
TABLE D	Season average 1939			Two-year aver. 1936–39	
Tobacco every year with manure, followed by rye cover crop	1.31	1.18	90.0	1006	196
Tobacco following 2 years of rye, disced when mature (1 crop of tobacco in 3 years)	1.46	1.27	87.0	1219	225
				Four-year aver. 1935–36–38–39	
Tobacco every year with manure, followed by rye cover crop	1.31	1.18	90.0	1312	330
Tobacco following tobacco, following rye disced when mature (2 crops of tobacco in 3 years)	1.26	1.09	86.5	1260	315
				1939	
Tobacco following 2 years of rye, disced when mature	1.46	1.27	87.0	1942	400
Tobacco following tobacco, following rye disced when mature	1.26	1.09	86.5	1594	326

greater returns per acre. The additions of manure in these rotations have had no significant effect on the content of organic matter, except possibly to increase the amount of the active portion in relation to the total amount. It is worthy of note, however, that the manure additions have invariably caused an increase in yield while at the same time the level of organic matter has been maintained. The literature reveals that the extent to which the organic matter and nitrogen contents of the soil are increased by repeated applications of practical quantities of manure is comparatively small, where intertilled crops are grown continuously. The important point is that the manure was apparently instrumental in maintaining the soil organic matter under increased crop production. In 20 years of rotations with and without manure, Peevy *et al.* (6) found that a significant loss of organic matter had occurred where the crop residues but no manure was returned, while this loss was greatly reduced where the rotations included the addition of manure.

A comparison of the 3-year rotations with continuous tobacco culture in Table 1, D, (4-year average) shows that where two crops of tobacco were followed by one crop of rye, both the organic matter content and the crop performance appeared to be in no way superior to continuous tobacco with manure. However, where tobacco followed two years of rye in the rotation (2-year average), both the total and the active organic matter were higher and the yield was increased over the continuous culture. Comparing the crop data of the two 3-year rotations of the year 1939, it is evident that the rotations having the higher organic matter in the soil produced the larger yield and greater returns per acre.

These experiments provide an indication of the potentiality of the rye crop in maintaining soil organic matter in flue-cured tobacco production. By applying barnyard manure or by returning the rye crops produced in a 2-year rotation, the total organic matter has not been significantly increased but the production of leaf was benefited extensively. Thus the value of the crop was definitely related to the amount of organic material which was added to the soil. This would appear to substantiate Albrecht's statement (1) that the value of organic matter lies in its dynamic nature and that it functions only as it is destroyed. As previously mentioned, too high a content of soil organic matter must be avoided because of the common undesirable qualities imparted to the leaf by the nitrogen which this organic matter slowly releases. It is rather then a matter of continuously supplying the soil with organic material of such a character that will decompose rapidly, providing the maximum amount of decomposition products early in the growing season and thus permitting the optimum development of the plant for bright leaf production.

SUMMARY

Results obtained from field plot experiments with flue-cured tobacco have shown that there is a general tendency toward higher yields of marketable leaf from plots containing larger amounts of soil organic matter. Where tobacco was grown every year the highest yields and the highest total organic matter were obtained where manure was applied and rye cover crops were grown in the intervals between seasons. The 2-year rotations in which tobacco was alternated with rye gave greater returns per acre than where tobacco was grown every year. The 3-year rotation in which tobacco followed two crops of rye provided much larger yields and returns per acre, and the soil organic matter was considerably higher than where tobacco was grown every year. In the 2-year rotations the application of manure caused no significant increase in the content of soil organic matter, but it did result in considerably increased yields of tobacco. At the same time the level of the organic matter was maintained under the increased production.

While the soil organic matter may not have been significantly increased by applying manure or by returning the rye crops produced in a two-year rotation, the production of leaf was enhanced by these practices. In this way the value of the crop was definitely related to the amount of organic material which was added to the soil.

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STUDIES ON SURFACE TAINT BUTTER

V. THE GROWTH OF *PSEUDOMONAS PUTREFACIENS* IN BUTTER[1]

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It is usually assumed that surface taint arises directly from the growth of specific micro-organisms in the affected butter. Many practical observations appear to support this hypothesis. On the other hand some practical experiences with the defect are not plausibly explained by such a theory. A reorientation of the evidence may not be out of place.

DISCUSSION OF THE LITERATURE

There is such an extensive literature on the growth of bacteria in butter that a comprehensive review is not possible within the space limits of this communication. Reasonably exhaustive bibliographies are readily available and need not be repeated here (5, 7, 8, 13, 14).

A perusal of this literature quickly reveals that the subject is in a chaotic state. There is a tendency for those who have worked with experimental butters churned in small lots to conclude that bacteria may grow extensively in butter, while measurements on commercial butters tend to point to the opposite conclusion. The reader is forced to conjecture if commercial butter structure is being duplicated experimentally.

The plate count has been the main measure of bacterial growth, and consideration has been given too infrequently to possible misleading results with this bacteriological tool. The determination of increases in metabolic products has driven no closer to the core of the problem because of the possibility of the independent action of enzymes after release from the bacterial cell. There is no unanimity of opinion as to the action of bacterial enzymes in butter. Some experimenters have even concluded that enzymes play no part whatever in butter deterioration.

The state of decomposition of the cream from which the butter was churned has often been ignored although it has been apparently shown that some bacteria may be less active in fermented- than in sweet-cream butter.

Rahn and Boysen (13) considered that even in sour-cream butter there are over 100 moisture droplets per bacterial cell while in pasteurized sweet-cream butter approximately 80% of the total moisture remains sterile.

Collins and Hammer (2) found evidence of bacterial migration in moisture channels in poorly-worked butters but were unable to demonstrate migration in the incorporated moisture of well-worked butters. These

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workers state that "studies in progress at the Iowa Agricultural Experiment Station suggest that in butter which becomes rancid through the action of bacteria there may be a rather rapid decrease in the bacterial content."

Jacobsen (8) claims that "the importance of micro-organisms in the development of butter flavours, other than rancidity and cheesiness, has not been well established".

The student of this problem is forcibly impressed with the overwhelming role in bacterially-induced butter deterioration played by gram-negative, rod-shaped, proteolytic bacteria which grow at low temperatures and are probably water-borne. Most, if not all, of the bacteria which have been reported as causing rancidity in butter are of this type. Herreid, Macy and Combs (5) in an extensive search for organisms producing cheese-like flavours in butter found gram-negative bacteria to predominate. Most of the lipolytic bacteria used by Hammer and Collins (4) were gram-negative rods. Nelson (11) related deterioration of butter caused by bacterial growth to "small thin rods".

Some disconcertingly high plate count increases in butter have been observed. Thus, for example, Long and Hammer (9) reported that the plate count of moderately-worked butter contaminated with "Culture A" increased in 2 days from approximately 100,000 to approximately 160,000,000. This is the approximate equivalent of 600,000 and 1,000,000,000 per ml. of butter moisture, represents an average increase of 1600 times, and equals the general maximum level of bacterial growth in liquid media (13). Again, Herreid *et al.* (5) reported plate counts of experimental, inoculated-cream butters after incubation to be as high as 500,000,000. This is the approximate equivalent of 3,000,000,000 per ml. of butter moisture.

When subjected to the criteria of the test-tube performance of bacteria, such bacterial growth requires a dispersion of moisture in butter that has not yet been reported by microscopists. If bacteria are able to grow to this extent in butter, it would seem that present popular theories of the physical structure of butter are in urgent need of drastic revision.

Hood and White (6) found the bacterial plate counts of commercial surface taint butters to vary from 90,000 to 160,000,000 with a tendency toward high counts. The same type of results were reported by Derby and Hammer (3) with a low of 11,000 and a high of over 300,000,000. The figures of Claydon and Hammer (1) vary from 125,000 to 9,200,000 for 3 samples of commercial putrid butter. The 21° C-2 day counts on experimental butters churned from creams inoculated with the 3 original butters varied from 6,800,000 to 105,000,000. Pont (12) quotes Loftus-Hills *et al* and Izterott as concluding that rabbito butters invariably contain large numbers of micro-organisms.

Although the evidence is most conflicting, the weight of it is to the effect that bacterially-induced butter flavour defects may occur in the absence of sufficient bacterial cells to account for the defect by any theory yet propounded. Those experiments in which plate counts indicated extensive bacterial growth in the butter yet remain to be integrated with any plausible theory of the distribution of moisture in butter.

EXPERIMENTAL

The Distribution of the Defect within a Churning

Practical experience seems to be that, if any portion of a churning of butter develops surface taint, the defect may be developed in any other portion of the same churning. Grading practices are in general use which depend upon this homogeneous distribution. It is not entirely unknown for the claim to be made that the grading sample developed surface taint while the rest of the churning did not. These exceptions, if they occur at all, appear to be sufficiently rare to be explained by difficulties of recognition, differences in incubation conditions and the temperamental-like characteristics of surface taint. Seldom, if ever, does the grading sample remain free of the defect while the remainder of the churning develops surface taint.

The distribution of the defect within the churning has the earmarks of a chemical adulteration of the butter rather than a bacterial contamination of, and subsequent growth in, a substance which, there is good reason to believe, is not a favourable medium for bacterial growth. Thus, the Australian workers believe that bacteria may elaborate a metabolic product from the residual milk solids in the equipment, particularly the churn, which, if incorporated into butter during manufacture, may give rise to rabbito.

Distribution of Butter Serum

At no time was it found possible to induce surface taint in experimental butter if the organism was introduced after the working process had proceeded sufficiently to prevent the even distribution of the causal agent throughout the butter. Nevertheless it was found, in confirmation of the report of Long and Hammer (9, 10) that the rate of appearance of the defect varied inversely with the degree of working. All other techniques which could logically be expected to exert an influence toward homogeneity of distribution of the causal agent favoured the defect. These statements apply, of course, only to procedures which do not interfere with the normal distribution of the serum in the butter.

The comment is not infrequently heard in this province that surface taint tends to attack the best-textured butters. On the other hand the Australian investigators report the tendency of rabbito to occur in butter of weak texture. The defect is undoubtedly related to other factors as well, such as the state of plant sanitation and the condition of the churning cream, and probably appears in Western Canadian butters in spite of good texture.

In the present study an effort was made to duplicate the thorough incorporation of moisture characteristic of the commercial butter of Western Canada. The experimental butters were worked with sterile wooden spatulas on soaked, sterilized boards. Throughout the course of the work these butters were examined by many experienced buttermakers and graders. At no time was it considered that the experimental butters were the equal of typical commercial butters in this regard. Therefore, phenomena that are influenced by the physical structure of butter should be interpreted with care in the case of butters worked by hand, at least until more is known regarding the physical nature of butter.

There is a wide-spread belief that butter cutters, particularly those with screw feeds, are sometimes responsible for the appearance of surface taint in the print butter. It is only necessary to observe the collection of pools of butter serum at various points while such machines are in operation to conclude that the distribution of the butter moisture is modified. Such manipulation of the butter opens up the possibility of bacterial growth in the disturbed serum as well as the contamination of this serum with a surface taint causal agent. The problem of the appearance of surface taint in manipulated butters is not simplified by the possible growth of many kinds of putrefactive bacteria in such butters with resulting defects which do not seem to be related to surface taint but are at times difficult to distinguish from it by the sense of smell.

This study included observations of several hundred samples of unworked butters and butters variously manipulated. It was most marked that, whenever manipulation caused the collection of serum in large aggregates, the trend of the keeping quality of the butter was toward that of liquid media. In all cases a mixed flora was present and in no case was it shown with certainty that the surface taint problem was in any way involved, although *Ps. putrefaciens* was known to be present in some samples.

Inoculation Technique

Experimental surface taint was produced during the course of these studies in over 300 churnings. It was found that it did not affect the course of the defect whether the cream was churned immediately after inoculation or whether churning was delayed over-night. It made no difference whether the inoculum was from a skim milk or broth culture or a water suspension of the organism from a slant culture. The defect was regularly produced when the butter wash-water was contaminated with the organism and when the butter was worked on wet contaminated boards.

Table 1.—Relation between contamination of butter wash-water and the grades of the resulting butters

	Source of inoculum							
	Broth culture					Skim milk culture		
Computed count*	0	78,000	780,000	7,800,000	78,000,000	780,000,000	11,000,000	110,000,000
Days after churning								
1½	C	C	C	C	?	?	?	?
2	C	C	C	ST(S)	ST(M)	ST(M)	ST(M)	ST(M)
3	C	C	C	ST(S)	ST(M)	ST(M)	ST(M)	ST(M)
4	C	C	?	ST(M)	ST(H)	ST(H)	ST(H)	ST(H)
6	C	C	C	C	ST(M)	ST(M)	ST(S)	ST(M)

C—Clean; ?—Questionable ST; ST—Surface Taint; H—Heavy; M—Moderate; S—Slight.

* The total number of cells of *Ps. putrefaciens*, based on the plate count, in the wash-water used to wash the butter churned from 1 pint of cream.

Nevertheless, there is a minimum inoculation necessary for the production of the defect. Table 1 shows the relation between the plate counts of the inoculated butter wash-water and the grading of the butters.

It was not possible to produce experimental surface taint by wrapping normal butters in parchment soaked in broth cultures of *Ps. putrefaciens*. This confirms the experience of Derby and Hammer (3) who were unable so to inoculate finished, normal butter that surface taint resulted.

These data support the theory that surface taint has its origin directly related to the growth of the bacterium in the butter. They are equally in support of the theory that the defect arises from enzyme action. In any case thorough incorporation and even distribution of the bacterium in the butter appear necessary.

Table 2.—Plate counts of commercial surface taint butters

Sample no.	Agar*	Nutrient gelatin	Sample no.	Agar*	Nutrient gelatin
1	>5,000,000	>5,000,000	21	5,000,000	5,960,000
2	910,000	1,000,000	22	153,000	148,000
3	224,000	700	24	450,000	430,000
5	15,300	9,000	25	372,000	2,190,000
6	259,000	173,000	26	333,000	279,000
7	51,000	Spreaders	27	>3,000,000	>3,000,000
8	700,000	10,000	28	>3,000,000	6,580,000
9	2,270,000	3,150,000	29	3,300,000	1,150,000
10	103,000	1,040,000	30	>3,000,000	4,770,000
11	30,000	10,000	31	>3,000,000	Liquefied
12	10,000	16,000	32	6,800,000	49,000
13	9,000	20,000	33	200,000	147,000
14	81,000	218,000	34	3,840,000	Liquefied
15	< 1,000	11,000	35	>3,000,000	4,200,000
16	< 1,000	42,000	36	>3,000,000	2,300,000
17	22,000	26,000	37	1,270,000	310,000
18	16,300	300	38	263,000	214,000
19	600	100	39	90,000	48,000
20	800	100	40	1,050,000	105,000

* Tryptone-glucose-meat-extract-2% skim milk agar.

The Plate Counts of Commercial Surface Taint Butters

The plate counts (Table 2) of 38 samples of commercial surface taint butters, mainly from Alberta and all from Western Canada, varied from 100 to over 6,000,000. Table 3 is included to show that the plate count range of 26 normal butters analysed concurrently with the defective butters varied over the same essential range although the normal butters tended toward lower counts.

This confirms the prior work of Hood and White and of Hammer and his co-workers. Nevertheless, the counts reported in Table 2 are much lower on the average than those reported heretofore. Most of these butters had been held for only short periods before analysis and had not been frozen. It is, therefore, unlikely that low temperatures were responsible for the death of considerable numbers of surface taint bacteria.

TABLE 3.—PLATE COUNTS OF NORMAL COMMERCIAL BUTTERS

Sample no.	Agar	Nutrient gelatin	Sample no.	Agar	Nutrient gelatin
1	>3,000,000	>3,000,000	14	2,200	1,200
2	>3,000,000	2,420,000	15	20,000	15,200
3	5,000	1,000	16	19,000	2,500
4	2,000	6,000	17	7,100	5,300
5	63,000	330,000	18	2,500	1,400
6	37,000	300,000	19	4,900	1,000
7	50,000	2,000	20	155,000	84,000
8	1,000	2,000	21	25,000	Liquefied
9	17,000	5,000	22	62,000	50,000
10	1,200,000	1,720,000	23	29,000	< 1,000
11	2,700	6,800	24	280,000	< 1,000
12	10,500	42,000	25	215,000	Liquefied
13	400	500	26	1,300	20,000

Two important considerations arise from these data. If it is assumed that the plate counts constitute reasonable estimates of the numbers of bacteria in these butters, then there are too few bacteria in many of these samples to account for the production of the taint through bacterial growth. If the plate counts are not representative of the bacterial populations, they are misleading and conclusions should not be based on them.

The second point is that isolations from the plates showed that bacteria producing surface taint constituted small minority populations, even in those butters in which their presence was proved. Such a view, of course, assumes this technique to be an adequate measure of surface taint bacteria.

This evidence does not point, therefore, in the direction of the extensive growth of bacteria in butter as being responsible for commercial surface taint.

NUMBER OF CELLS AND ODOUR PRODUCTION BY *Ps. putrefaciens*

It was found that a minimum Breed count of 50,000,000 cells of *Ps. putrefaciens* was required for the reduction of litmus in milk. Since odour production was not observed prior to litmus reduction, it follows that extensive bacterial growth precedes odour production in litmus milk.

By means of plate counts on tryptone-glucose-beef-extract-2% skim milk agar, bacterial numbers were followed in 5 inoculated-cream butters held at 10° C. to 15° C. for periods up to 23 days. The defect appeared in less than 3 days in all butters. Examination of Table 4 shows that the 3-day counts varied from 132,000 to 1,500,000 per ml. of butter. This is the approximate equivalent of only 792,000 and 9,000,000 per ml. of butter serum. In the present studies the highest plate count of inoculated-cream butters observed at any time after manufacture was 50,000,000. This is the approximate equivalent of 300,000,000 per ml. of butter serum. Many isolations were made from these plates and the cultures were considered to be *Ps. putrefaciens* if they were gram-negative rods which reduced litmus milk rapidly without change of reaction, proteolysed the upper layer of the milk in the characteristic manner and produced the characteristic sweaty feet odour. By these criteria *Ps. putrefaciens* constituted from 40% to 100% of the bacterial populations in these butters.

TABLE 4.—PLATE COUNTS OF EXPERIMENTAL SURFACE TAINT BUTTERS

Days after churning	Butter number				
	1	2	3	4	5
1	800,000	22,000	40,000	320,000	13,100
2	265,000	153,000			
3			132,000	280,000	1,500,000
4	340,000	80,000			
5			100,000	710,000	93,000
6	600,000	330,000			
8	1,000,000	2,000,000			
10			3,200,000	9,600,000	16,800,000
12	3,400,000	700,000			
16			13,000,000	7,500,000	10,000,000
20	14,500,000	17,500,000			
23			4,800,000	15,100,000	3,500,000

If these plate counts are representative of the bacterial contents of the butters, it is difficult to relate the appearance of the defect to the growth of the bacteria in the butters.

SIZE OF INOCULUM

It is one of the puzzling features of commercial surface taint that bacteria producing this defect have not yet been found in large numbers in the affected butters. Clayden and Hammer reported difficulty in inducing growth initiation from small inocula of *Ps. putrefaciens* . This may explain the apparent absence of surface-taint-producing bacteria in so many of the defective butters.

Throughout the present study the difficulty of growing *Ps. putrefaciens* has not been impressive and the organism has been regarded as one growing with ease on most of the common laboratory media.

On the other hand it has been found that *Ps. putrefaciens* initiates growth in litmus milk only when comparatively large inocula are used. This is illustrated by the data in Table 5. In this experiment 100 ml. of litmus milk or nutrient broth were inoculated with 1 ml. of a water suspension of cells from an agar slant culture of the organism. The inoculated media were incubated at 25° C. and plate counts on 4 media were deter-

TABLE 5.—PLATE COUNTS OF *Ps. putrefaciens* GROWN IN LITMUS MILK AND NUTRIENT BROTH AT 25° C.

Plating medium	Plate incubation temperature	Skim milk culture			Broth culture		
		0 hrs.	12 hrs.	24 hrs.	0 hrs.	12 hrs.	24 hrs.
TGEM agar	25° C.	91,000	<10,000	<10,000	104,000	47,000,000	78,000,000
Nutrient agar	25° C.	76,000	<10,000	<10,000	129,000	10,700,000	92,000,000
Nutrient gelatin	10–15° C.	205,000	<10,000	<10,000	261,000	32,600,000	79,000,000
Beef infusion agar	25° C.	22,500	<10,000	<10,000			

mined at various intervals. The nutrient gelatin plates were incubated at 10° C. to 15° C. for 5 days while the rest of the plates were counted after 5 days at 25° C. Inocula resulting in plate counts at zero time of approximately 100,000 were insufficiently large to induce initiation of growth in the litmus milk and the organism was not recovered from the milk after 12 hours of incubation. That the organism did not grow in the litmus milk is also shown by the fact that the litmus did not reduce over a period of 5 days.

In a previous paper in this series it was suggested that the E_h and poising capacity of milk may act antagonistically toward growth of *Ps. putrefaciens*.

Germicides

Surface taint did not develop in butters churned from cream containing cells of *Ps. putrefaciens* which had been treated with such odourless germicides as mercuric chloride and acid. It seems that the presence of the living bacteria in the butter is necessary for the development of the defect.

Oxygen

One of the outstanding characteristics of surface taint, which was first observed by Marker in 1919, is that freshly-cut surfaces of affected butter are free from the odour. The characteristic surface taint odour appears on these surfaces when the butter is held for a few hours at room temperature. It has been usual to assume that the incubation period permits bacterial growth on the surface of the butter, thus producing the defect.

In a study of this point it was observed that freshly-cut cubes of experimental surface taint butter did not develop the defect when held for $2\frac{1}{2}$ days at 10° C. to 15° C. in a rarefied atmosphere freed of O_2 by alkaline pyrogallol. This would seem to point to oxidation of a reduced chemical as the key to the surface peculiarities of this defect. However, inoculated agar slopes failed to exhibit growth when subjected to the same treatment. Upon removal from the O_2-free air for 12 hours, surface taint appeared on the butter surfaces and growth was evident on the agar slants.

At least two interpretations of these data are possible. The lack of oxygen may have maintained a compound in a reduced state or it may have inhibited bacterial growth in the surface layers of the butter.

It is a common observation that the defect will appear on freshly-cut surfaces of affected butter held over-night at room temperature. The most rapid appearance of the defect on freshly-cut surfaces of experimental butter observed in the course of the present studies took place within 3 hours. It seems probable that extensive growth did not occur on these surfaces in this short time and that the phenomenon is related to the oxidation of a reduced compound that is already present within the butter.

pH

The relation of the development of surface taint to the butter serum pH (15) largely supports the theory of the growth of the organism in the butter. It does not, however, completely rule out chemical possibilities. If it is possible to remove the defect from affected butter by the adjustment of the pH of the butter, as is believed by some practical buttermakers but for which it has been impossible to gain experimental substantiation, then a chemical rather than a biological reaction would seem to be involved.

DISCUSSION

Common experience leaves no room for doubt that butter and liquid media, such as, for instance, plastic cream, differ markedly in their respective keeping-qualities. This observation is incompatible with the conception that bacterial growth in butter may equal in rate and quantity bacterial growth in liquid media.

There seem to be no available studies relating the phenomena of bacterial dissociation to apparent bacterial growth in butter. Dissociation possibilities require to be ruled out before plate count criteria may be adequately evaluated.

The question of the growth of bacteria in butter is still largely in the realm of conjecture. No less obscure is the mechanism of surface taint production. The bulk of the evidence is of an empirical nature and most confusing. Precise interpretations are a matter for the future.

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DIGESTIBILITY STUDIES WITH RUMINANTS

IX. ASSOCIATIVE DIGESTIBILITY OF GRAINS: WHEAT BRAN, GLUTEN FEED AND SOYBEAN OIL MEAL[1]

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The total digestible nutrients and digestible protein in a grain mixture are calculated from the values for the individual feeds. It is assumed that such values undergo no change when the individual feeds are incorporated into mixtures. To verify or disprove this assumption digestibility studies on a number of grains were undertaken. Their contents in digestible nutrients and digestible protein were determined by digestion trials with steers, using hay as a basal ration. Various combinations of these grains were then made up and the digestible nutrients of the resulting mixed rations were calculated from the values of the individual grains. These grain mixtures were then fed to steers in digestion trials with hay as a basal ration, and their actual content in digestible nutrients determined. These latter values will be called "observed values", whereas those calculated from the values of the individual feeds will be called "calculated values".

Six feeds were used, namely, barley, oats, linseed oil meal, wheat bran, gluten feed, and soybean oil meal. The results with the first three of these have been published (1). In this paper are presented the results with bran, gluten feed, soybean oil meal and a mixture of equal parts by weight of all three of these feeds. Each concentrate, as well as the mixture, was fed in digestion trials with hay as the basal ration. Since a trial with hay alone was necessary, this made a total of five rations.

The experiment, therefore, was set up in the form of a 5×5 randomized Latin square, the 5 rations being fed in 5 periods to 5 steers. Each period lasted 24 days, 12 of which were preliminary and 12 collection. The schedule is shown in Table 1.

With regard to the details concerning the animals and the feeds, the animals were grade Shorthorn steers, numbered H, 1, 2, 3, and 4. Their mean weights were 610, 510, 497, 491, and 574 kilograms respectively. Animal H was $3\frac{1}{2}$ years old and the remainder were $2\frac{1}{2}$. The hay was mostly clover. Its botanical composition is given in Table 3 in the Appendix. The wheat bran and gluten feed were commercial feedstuffs purchased in the usual manner. The soybean oil meal was produced by the solvent process.

All the experimental data will be found in the Appendix in Tables 3 to 9. The essential facts have been summarized in Table 2. In this table a comparison has been made of the means of the calculated coefficients of the grain mixture and of the means of the observed coefficients. A com-

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parison has also been made of the calculated total digestible nutrients in the dry matter and the observed total digestible nutrients. It is evident that the two series of values are almost identical. In some cases *t* values have been calculated, but the differences were obviously in no way significant.

Certain points in the tables in the Appendix deserve mention. It is evident from Tables 6 and 7 that there was a statistically significant difference between periods. Therefore, some of the variance occurring in calculating the *t* values was due to the effect of period, and its elimination would reduce the standard error of the difference. When, however, the two series of values were examined statistically by the method of paired differences, the conclusions were the same. The differences between the means, where differences occurred, were so small that the reduction of the error had no effect on the results. It is concluded, therefore, that for the concentrates—wheat bran, gluten feed, and soybean oil meal—there was no effect of associative digestibility.

SUMMARY

Using 5 grade Shorthorn steers the coefficients of digestibility were determined for wheat bran, gluten feed, soybean oil meal, and a mixture consisting of equal parts of these grains. From the values of the individual grains a calculation was made of the coefficients of digestibility of the grain mixture. These calculated values were practically identical with the observed values determined by digestibility trials.

It was concluded that there was no effect of associative digestibility among these three grains.

ACKNOWLEDGMENT

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APPENDIX

TABLE 1.—FEEDING SCHEDULE

Period	Feeds	Kilograms per animal per day				
		Animal H	Animal 1	Animal 2	Animal 3	Animal 4
1	Hay	4	4	4	6.5	4
	Bran	3	–	1	–	–
	Gluten feed	–	3	1	–	–
	Soybean oil meal	–	–	1	–	3
2	Hay	4	4	6	4	4
	Bran	1	3	–	–	–
	Gluten feed	1	–	–	–	3
	Soybean oil meal	1	–	–	3	–
3	Hay	6	4	4	4	4
	Bran	–	1	–	–	–
	Gluten feed	–	1	–	3	–
	Soybean oil meal	–	1	3	–	–
4	Hay	4	4	4	4	6
	Bran	–	–	3	1	–
	Gluten feed	3	–	–	1	–
	Soybean oil meal	–	2	–	1	–
5	Hay	4	6	3	3	4
	Bran	–	–	–	2	1
	Gluten feed	–	–	2	–	1
	Soybean oil meal	3	–	–	–	1

TABLE 2.—MEAN* COEFFICIENTS OF DIGESTIBILITY AND MEAN TOTAL DIGESTIBLE NUTRIENTS IN GRAIN MIXTURE†

	Coefficients of digestibility in per cent					T.D.N. in dry matter in per cent
	Dry matter	Organic matter	Nitrogen	Ether extract	Total carbo-hydrates	
Calculated‡	81.6	82.7	87.2	83.4	79.9	81.02
Observed§	81.3	82.5	87.2	82.8	79.8	80.81
Difference	0.3	0.2	0	0.6	0.1	0.21
t††	0.206	0.136		0.276		0.143

* Means derived from 5 individual values.
† Grain mixture composed of equal parts of wheat bran, gluten feed and soybean oil meal.
‡ The calculated values for each period were determined from the coefficients of digestibility of the individual grains for that period. These were applied to the quantity of each grain in the grain mixture.
§ Determined by digestion trials.
†† Necessary "t" at P of 0.05 = 2.306.

TABLE 3.—BOTANICAL COMPOSITION OF HAY

Component	Per cent present	
	Top of mow	Bottom of mow
Red and alsike clover	72.6	75.8
Alfalfa	17.9	20.8
Grass	7.9	3.2
Weeds	1.6	0.2

TABLE 4.—CHEMICAL COMPOSITION OF FEEDING STUFFS

Feed	Period no.	Moisture	In dry matter				
			Ash	Protein*	Ether extract	Crude fibre	N-free extract
		%	%	%	%	%	%
Hay	1	13.95	7.62	13.18	1.94	33.97	43.29
	2	14.69	7.85	13.51	2.21	34.65	41.78
	3	14.54	7.73	13.49	1.97	35.06	41.75
	4	14.30	8.34	14.18	2.25	33.70	41.53
	5	13.10	7.30	12.25	2.09	37.54	40.82
	Means	14.12	7.77	13.32	2.09	34.98	41.83
Wheat bran	Triple†	13.15	6.13	18.65	5.09	11.92	58.21
	1	12.83	5.92	18.85	4.95	11.62	58.66
	2 and 3	12.51	5.83	18.65	4.78	11.82	58.92
	4 and 5	12.76	5.92	18.52	5.16	11.54	58.86
	Means	12.81	5.95	18.67	5.00	11.73	58.66
Gluten feed	1†	11.24	5.56	33.95	3.11	6.61	50.77
	2 and 3	11.52	4.77	33.95	3.44	6.95	50.89
	4 and 5	10.34	5.31	32.78	3.43	7.30	51.18
	Means	11.03	5.21	33.56	3.33	6.95	50.95
Soybean oil meal	1†	12.30	6.60	47.62	1.33	6.06	38.39
	2 and 3	12.45	6.68	47.47	1.34	5.70	38.81
	4 and 5	12.36	6.66	47.52	1.34	5.97	38.51
	Means	12.37	6.65	47.54	1.34	5.91	38.57

* Protein factors were: Hay—6.25; Bran—6.31; Gluten feed—6.25; Soybean oil meal—5.71.

† For the triple grain mixture a separate sample was taken for bran, but for gluten feed and soybean oil meal samples were the same as for period 1.

TABLE 5.—CALCULATION OF COEFFICIENTS OF DIGESTIBILITY

(Collection period of 12 days: weights in kilogrammes: coefficients in per cent)

	Original weight	Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N-free extract
PERIOD I							
Animal H							
In hay	48.000	41.304	38.157	0.871	0.801	14.031	17.881
In bran	36.000	31.381	29.523	0.938	1.553	3.646	18.408
In feces	129.757	28.664	25.499	0.536	0.771	10.488	10.898
Dig. from hay		21.561	20.719	0.494	0.340	6.005	11.319
Dig. from bran		22.460	21.462	0.779	1.243	1.184	14.072
Coeff. of bran		71.6	72.7	83.0	80.0	32.5	76.4
Animal 1							
In hay	48.000	41.304	38.157	0.871	0.801	14.031	17.881
In gluten feed	33.000	29.291	27.662	1.591	0.911	1.936	14.871
In feces	122.296	22.652	19.766	0.556	0.548	8.341	7.603
Dig. from hay		21.561	20.719	0.494	0.340	6.005	11.319
Dig. from gluten feed		26.382	25.334	1.412	0.824	1.621	13.830
Coeff. of gluten feed		90.1	91.6	88.7	90.5	83.7	93.0

TABLE 5.—CALCULATION OF COEFFICIENTS OF DIGESTIBILITY—*Continued*

(Collection period of 12 days: weights in kilogrammes: coefficients in per cent)

	Original weight	Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N-free extract
PERIOD 1—*Con.*							
Animal 2							
In hay	49.000	41.304	38.157	0.871	0.801	14.031	17.881
In bran	12.000	10.422	9.783	0.308	0.530	1.242	6.067
In gluten feed	12.000	10.651	10.059	0.579	0.331	0.704	5.408
In soybean meal	12.000	10.524	9.829	0.878	0.140	0.638	4.040
In total grain	36.000	31.597	29.671	1.765	1.001	2.584	15.515
In feces	126.283	24.404	21.471	0.573	0.586	8.873	8.511
Dig. from hay		21.561	20.719	0.494	0.340	6.005	11.319
Dig. from grain		26.938	25.638	1.569	0.876	1.737	13.566
Coeff. of grain		85.3	86.4	88.9	87.5	67.2	87.4
Animal 3							
In hay	78.000	67.119	62.005	1.415	1.302	22.800	29.056
In feces	183.492	31.494	27.696	0.614	0.746	13.187	9.936
Coeff. of dig.		53.1	55.3	56.6	42.7	42.2	65.8
Animal 4							
In hay	48.000	41.304	38.157	0.871	0.801	14.031	17.881
In soybean meal	36.000	31.572	29.488	2.633	0.420	1.913	12.120
In feces	143.313	22.892	20.037	0.624	0.627	8.477	7.202
Dig. from hay		21.561	20.719	0.494	0.340	6.005	11.319
Dig. from meal		28.423	26.889	2.386	0.254	1.462	11.480
Coeff. of meal		90.0	91.2	90.6	60.5	76.4	94.7
PERIOD 2							
Animal H							
In hay	48.000	40.949	37.735	0.886	0.905	14.189	17.108
In triple mixture	36.000	31.597	29.671	1.765	1.001	2.584	15.515
In feces	135.979	25.227	22.308	0.579	0.656	9.150	9.024
Dig. from hay		21.375	20.490	0.502	0.384	6.073	10.829
Dig. from grain		25.944	24.608	1.570	0.866	1.550	12.770
Coeff. of grain		82.1	82.9	89.0	86.5	60.0	82.3
Animal 1							
In hay	48.000	40.949	37.735	0.886	0.905	14.189	17.108
In bran	36.000	31.496	29.660	0.931	1.506	3.723	18.557
In feces	141.308	29.587	26.495	0.574	0.754	10.784	11.569
Dig. from hay		21.375	20.490	0.502	0.384	6.073	10.829
Dig. from bran		21.483	20.410	0.741	1.273	1.055	13.267
Coeff. of bran		68.2	68.8	79.6	84.5	28.3	71.5
Animal 2							
In hay	72.000	61.423	56.601	1.329	1.357	21.283	25.663
In feces	164.597	29.591	26.262	0.558	0.695	12.671	9.602
Coefficient		51.8	53.6	58.0	48.8	40.5	62.6
Animal 3							
In hay	48.000	40.949	37.735	0.886	0.905	14.189	17.108
In soybean oil meal	36.000	31.518	29.413	2.620	0.422	1.797	12.232
In feces	148.884	23.843	21.123	0.623	0.596	9.389	7.575
Dig. from hay		21.375	20.490	0.502	0.384	6.073	10.829
Dig. from meal		27.249	25.535	2.381	0.347	0.524	10.936
Coeff. of meal		86.5	86.8	90.9	82.2	29.2	89.4
Animal 4							
In hay	48.000	40.949	37.735	0.886	0.905	14.189	17.108
In gluten feed	36.000	31.853	30.334	1.731	1.096	2.214	16.210
In feces	134.406	23.341	20.818	0.559	0.579	8.968	8.041
Dig. from hay		21.375	20.490	0.502	0.384	6.073	10.829
Dig. from gluten feed		28.086	26.761	1.556	1.038	1.362	14.448
Coeff. of gluten feed		88.2	88.2	89.9	94.7	61.5	89.1

TABLE 5.—CALCULATION OF COEFFICIENTS OF DIGESTIBILITY—*Continued*

(Collection period of 12 days: weights in kilogrammes: coefficients in per cent)

	Original weight	Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N-free extract
PERIOD 3							
Animal H							
In hay	72.000	61.531	56.775	1.328	1.212	21.573	25.689
In feces	149.876	29.209	25.943	0.549	0.789	12.186	9.636
Coefficient		52.5	54.3	58.7	34.9	43.5	62.5
Animal 1							
In hay	48.000	41.021	37.850	0.885	0.808	14.382	17.126
In triple mixture	36.000	31.597	29.671	1.765	1.001	2.584	15.515
In feces	155.950	25.947	22.953	0.621	0.682	9.611	9.035
Dig. from hay		21.413	20.553	0.502	0.343	6.155	10.841
Dig. from grain		25.258	24.015	1.527	0.784	1.200	12.765
Coeff. of grain		79.9	80.9	86.5	78.3	46.4	82.3
Animal 2							
In hay	48.000	41.021	37.850	0.885	0.808	14.382	17.126
In soybean meal	36.000	31.518	29.413	2.620	0.422	1.797	12.232
In feces	139.119	23.269	20.472	0.638	0.652	8.554	7.418
Dig. from hay		21.413	20.553	0.502	0.343	6.155	10.841
Dig. from meal		27.857	26.238	2.365	0.235	1.470	11.099
Coeff. of meal		88.4	89.2	90.3	55.7	81.8	90.7
Animal 3							
In hay	48.000	41.021	37.850	0.885	0.808	14.382	17.126
In gluten feed	36.000	31.853	30.334	1.731	1.096	2.214	16.210
In feces	136.100	24.428	21.545	0.612	0.642	8.767	8.506
Dig. from hay		21.413	20.553	0.502	0.343	6.155	10.841
Dig. from gluten feed		27.033	26.086	1.502	0.919	1.674	13.989
Coeff. of gluten feed		84.9	86.0	86.8	83.9	75.6	86.3
Animal 4							
In hay	48.000	41.021	37.850	0.885	0.808	14.382	17.126
In bran	36.000	31.496	29.660	0.931	1.506	3.723	18.557
In feces	184.937	28.022	24.788	0.570	0.673	9.791	10.912
Dig. from hay		21.413	20.553x	0.502	0.343	6.155	10.841
Dig. from bran		23.082	22.169	0.744	1.298	2.159	13.930
Coeff. of bran		73.3	74.7	79.9	86.2	58.0	75.1
PERIOD 4							
Animal H							
In hay	48.000	44.136	37.705	0.933	0.926	13.863	17.084
In gluten feed	36.000	32.278	30.564	1.693	1.107	2.356	16.520
In feces	128.971	24.525	21.374	0.641	0.706	8.258	8.498
Dig. from hay		21.473	20.474	0.529	0.393	5.933	10.814
Dig. from gluten feed		27.416	26.421	1.456	0.934	2.028	14.292
Coeff. of gluten feed		84.9	86.4	86.0	84.4	86.1	86.5
Animal 1							
In hay	48.000	41.136	37.705	0.933	0.926	13.863	17.084
In soybean meal	24.000	21.034	19.633	17.51	0.282	1.256	8.100
In feces	128.297	22.903	19.949	0.589	0.595	8.710	7.155
Dig. from hay		21.473	20.474	0.529	0.393	5.933	10.814
Dig. from meal		17.794	16.915	1.566	0.220	0.476	7.215
Coeff. of meal		84.6	86.2	89.4	78.0	37.9	89.1

TABLE 5.—CALCULATION OF COEFFICIENTS OF DIGESTIBILITY—*Concluded*

(Collection period of 12 days: weights in kilogrammes: coefficients in per cent)

	Original weight	Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N-free extract
PERIOD 4							
Animal 2							
In hay	48.000	41.136	37.705	0.933	0.926	13.863	17.084
In bran	36.000	31.406	29.547	0.922	1.621	3.624	18.486
In feces	154.463	29.726	26.429	0.605	0.770	10.312	11.581
Dig. from hay		21.473	20.474	0.529	0.393	5.933	10.814
Dig. from bran		21.343	20.349	0.721	1.384	1.242	13.175
Coeff. of bran		68.0	68.9	78.2	85.4	34.3	71.3
Animal 3							
In hay	48.000	41.136	37.705	0.933	0.926	13.863	17.084
In triple mixture	36.000	31.597	29.671	1.765	1.001	2.584	15.515
In feces	154.631	26.637	23.433	0.647	0.735	9.030	9.741
Dig. from hay		21.473	20.474	0.529	0.393	5.933	10.814
Dig. from grain		24.623	23.469	1.522	0.799	1.484	12.044
Coeff. of grain		77.9	79.1	86.2	79.8	57.4	77.6
Animal 4							
In hay	72.000	61.704	56.558	1.400	1.388	20.794	25.626
In feces	199.068	29.966	26.142	0.597	0.803	11.911	9.820
Coefficient		51.4	53.8	57.4	42.1	42.7	61.7
PERIOD 5							
Animal H							
In hay	48.000	41.712	38.667	0.818	0.872	15.659	17.027
In soybean meal	36.000	31.550	29.449	2.626	0.423	1.884	12.150
In feces	162.590	24.070	21.201	0.661	0.631	9.091	7.527
Dig. from hay		21.774	20.996	0.464	0.370	6.702	10.778
Dig. from meal		27.418	25.919	2.319	0.294	1.750	10.872
Coef. of meal		86.9	88.0	88.3	69.5	92.9	89.5
Animal 1							
In hay	72.000	62.568	58.001	1.226	1.308	23.488	25.540
In feces	166.525	29.939	26.445	0.577	0.739	12.865	9.254
Coefficient		52.1	54.4	52.9	43.5	45.2	63.8
Animal 2							
In hay	36.000	31.284	29.000	0.613	0.654	11.744	12.770
In gluten feed	24.000	21.518	20.375	1.128	0.738	1.571	11.013
In feces	99.889	18.003	15.807	0.441	0.502	6.477	6.058
Dig. from hay		16.330	15.747	0.348	0.277	5.026	8.083
Dig. from gluten feed		18.469	17.821	0.952	0.613	1.812	9.642
Coeff. of gluten feed		85.8	87.5	84.4	83.1	115.3	87.6
Animal 3							
In hay	30.000	26.070	24.167	0.511	0.545	9.787	10.642
In bran	20.000	17.448	16.415	0.512	0.900	2.013	10.270
In feces	107.374	17.448	15.478	0.348	0.429	6.166	6.749
Dig. from hay		13.609	13.123	0.290	0.231	4.189	6.736
Dig. from bran		12.461	11.981	0.385	0.785	1.445	7.427
Coeff. of bran		71.4	73.0	75.2	87.2	71.8	72.3
Animal 4							
In hay	48.000	44.712	38.667	0.818	0.872	15.659	17.027
In triple mixture	36.000	31.597	29.671	1.765	1.001	2.584	15.515
In feces	162.286	25.770	22.605	0.608	0.685	8.999	9.190
Dig. from hay		21.774	20.996	0.464	0.370	6.702	10.778
Dig. from mixture		25.765	24.737	1.511	0.818	2.542	12.574
Coeff. of mixture		81.5	83.4	85.6	81.7	98.4	81.0

TABLE 6.—SUMMARY OF COEFFICIENTS OF DIGESTIBILITY

(Coefficients in per cent; Animal numbers shown by brackets)

Nutrient	Period No.	Coefficients of digestibility					
		Hay	Calculated from hay rations				Means
			Bran	Gluten feed	Soybean oil meal	Triple mixture	
Dry matter	1	53.1 (3)	71.6 (H)	90.1 (1)	90.0 (4)	85.3 (2)	78.0
	2	51.8 (2)	68.2 (1)	88.2 (4)	86.5 (3)	82.1 (H)	75.4
	3	52.5 (H)	73.3 (4)	84.9 (3)	88.4 (2)	79.9 (1)	75.8
	4	51.4 (4)	68.0 (2)	84.9 (H)	84.6 (1)	77.9 (3)	73.4
	5	52.1 (1)	71.4 (3)	85.8 (2)	86.9 (H)	81.5 (4)	75.5
	Means	52.2	70.5	86.8	87.3	81.3	75.62
Organic matter	1	55.3 (3)	72.7 (H)	91.6 (1)	91.2 (4)	86.4 (2)	79.4
	2	53.6 (2)	68.8 (1)	88.2 (4)	86.8 (3)	82.9 (H)	76.1
	3	54.3 (H)	74.7 (4)	86.0 (3)	89.2 (2)	80.9 (1)	77.0
	4	53.8 (4)	68.9 (2)	86.4 (H)	86.2 (1)	79.1 (3)	74.9
	5	54.4 (1)	73.0 (3)	87.5 (2)	88.0 (H)	83.4 (4)	77.3
	Means	54.3	71.6	87.9	88.3	82.5	76.93
Nitrogen	1	56.6 (3)	83.0 (H)	88.7 (1)	90.6 (4)	88.9 (2)	81.6
	2	58.0 (2)	79.6 (1)	89.9 (4)	90.9 (3)	89.0 (H)	81.5
	3	58.7 (H)	79.9 (4)	86.8 (3)	90.3 (2)	86.5 (1)	80.4
	4	57.4 (4)	78.2 (2)	86.0 (H)	89.4 (1)	86.2 (3)	79.4
	5	52.9 (1)	75.2 (3)	84.4 (2)	88.3 (H)	85.6 (4)	77.3
	Means	56.7	79.2	87.2	89.9	87.2	80.04
Ether extract	1	42.7 (3)	80.0 (H)	90.5 (1)	60.5 (4)	87.5 (2)	72.2
	2	48.8 (2)	84.5 (1)	94.7 (4)	82.2 (3)	86.5 (H)	79.3
	3	34.9 (H)	86.2 (4)	83.9 (3)	55.7 (2)	78.3 (1)	67.8
	4	42.1 (4)	85.4 (2)	84.4 (H)	78.0 (1)	79.8 (3)	73.9
	5	43.5 (1)	87.2 (3)	83.1 (2)	69.5 (H)	81.7 (4)	73.0
	Means	42.4	84.7	87.3	69.2	82.8	73.26
Crude fibre	1	42.2 (3)	32.5 (H)	83.7 (1)	76.4 (4)	67.2 (2)	60.4
	2	40.5 (2)	28.3 (1)	61.5 (4)	29.2 (3)	60.0 (H)	43.9
	3	43.5 (H)	58.0 (4)	75.6 (3)	81.8 (2)	46.4 (1)	61.1
	4	42.7 (4)	34.3 (2)	86.1 (H)	37.9 (1)	57.4 (3)	51.7
	5	45.2 (1)	71.8 (3)	115.3 (2)	92.9 (H)	98.4 (4)	84.7
	Means	42.8	45.0	84.4	63.6	65.9	60.35
N-free extract	1	65.8 (3)	76.4 (H)	93.0 (1)	94.7 (4)	87.4 (2)	83.5
	2	62.6 (2)	71.5 (1)	89.1 (4)	89.4 (3)	82.3 (H)	79.0
	3	62.5 (H)	75.1 (4)	86.3 (3)	90.7 (2)	82.3 (1)	79.4
	4	61.7 (4)	71.3 (2)	86.5 (H)	89.1 (1)	77.6 (3)	77.2
	5	63.8 (1)	72.3 (3)	87.6 (2)	89.5 (H)	81.0 (4)	78.8
	Means	63.3	73.3	88.5	90.7	82.1	79.58
Total carbo-hydrates (indirectly)	1	55.4 (3)	69.2 (H)	91.9 (1)	92.2 (4)	84.6 (2)	
	2	52.6 (2)	64.3 (1)	85.8 (4)	81.7 (3)	79.1 (H)	
	3	53.8 (H)	72.2 (4)	85.0 (3)	89.6 (2)	77.2 (1)	
	4	53.2 (4)	65.2 (2)	86.5 (H)	82.2 (1)	74.7 (3)	
	5	54.9 (1)	72.2 (3)	91.0 (2)	89.9 (H)	83.5 (4)	
	Means	54.0	68.6	88.0	87.1	79.8	

ANIMAL MEANS

Animal	Dry matter	Organic matter	Nitrogen	Ether extract	Crude fibre	N-free extract
1	75.0	76.4	79.4	75.0	48.3	79.9
2	75.9	77.1	80.0	72.1	67.8	79.9
3	74.8	76.0	79.1	75.2	55.2	78.3
4	76.9	78.3	80.7	73.0	67.4	80.3
H	75.6	76.9	81.0	71.1	63.0	79.4

TABLE 7.—ANALYSIS OF VARIANCE OF COEFFICIENTS OF DIGESTIBILITY*

Nutrient	Variance due to	D/F	Sums of squares	Variance	$\frac{1}{2}\log_e V$	z compared with error
Dry matter	Ration	4	4341.94	1085.485	3.4949	3.1075
	Period	4	53.44	13.360	1.2961	0.9087
	Animal	4	13.87	3.468	0.6218	0.2344
	Error	12	26.04	2.170	0.3874	
	Total	24	4435.29			
Organic matter	Ration	4	4105.84	1026.460	3.4669	2.9607
	Period	4	55.26	13.815	1.3129	0.8067
	Animal	4	15.26	3.815	0.6695	0.1633
	Error	12	33.02	2.752	0.5062	
	Total	24	4209.38			
Nitrogen	Ration	4	3726.06	931.515	3.4184	3.8067
	Period	4	63.06	15.765	1.3789	1.7672
	Animal	4	13.26	3.315	0.5992	0.9875
	Error	12	5.52	0.460	$\bar{1}$.6117	
	Total	24	3807.90			
Ether extract	Ration	4	6939.15	1734.788	3.7293	1.9830
	Period	4	339.47	84.868	2.2205	0.4742
	Animal	4	64.35	16.088	1.3890	†
	Error	12	394.45	32.871	1.7463	
	Total	24	7737.42			
Crude fibre	Ration	4	5816.96	1454.24	3.6411	1.1803
	Period	4	4694.56	1173.64	3.5339	1.0731
	Animal	4	1419.76	354.94	2.9360	0.4752
	Error	12	1646.54	137.21	2.4608	
	Total	24	13577.82			
N-free extract	Ration	4	2570.24	642.560	3.2327	3.2701
	Period	4	110.04	27.510	1.6573	1.6947
	Animal	4	11.97	2.993	0.5481	0.5855
	Error	12	11.13	0.928	$\bar{1}$.9626	
	Total	24	2703.38			

† Variance for animal less than variance for error.
* z for $n_1 = 4$, $n_2 = 12$ at P of 0.05 = 0.5907.
z for $n_1 = 4$, $n_2 = 12$ at P of 0.01 = 0.8443.

TABLE 8—TOTAL DIGESTIBLE NUTRIENTS IN PERCENTAGE OF DRY MATTER

Period	Bran	Gluten feed	Soybean oil meal	Triple mixture
	%	%	%	%
1	73.12	89.75	85.94	84.41
2	69.63	86.94	81.90	81.28
3	75.47	84.64	84.42	78.74
4	70.06	85.24	81.46	77.34
5	74.68	87.20	84.08	82.30
Means	72.59	86.75	83.56	80.81

TABLE 9.—CALCULATION OF TOTAL DIGESTIBLE NUTRIENTS IN TRIPLE GRAIN MIXTURE FROM THE VALUES FOR THE INDIVIDUAL FATS

Period	Total digestible nutrients in dry matter				
	In bran*	In gluten feed	In soybean oil meal*	Total*	Total in 100 kg. of mixture
	kg.	kg.	kg.	kg.	kg.
1	7.621	9.559	9.044	26.224	83.00
2	7.257	9.260	8.619	25.136	79.55
3	7.865	9.015	8.884	25.764	81.54
4	7.302	9.079	8.573	24.954	78.98
5	7.783	9.288	8.849	25.920	82.03

* The grain mixture as fed consisted of 10.422 kg. of bran, 10.651 of gluten feed, and 10.524 of soybean oil meal, making a total of 31.597.